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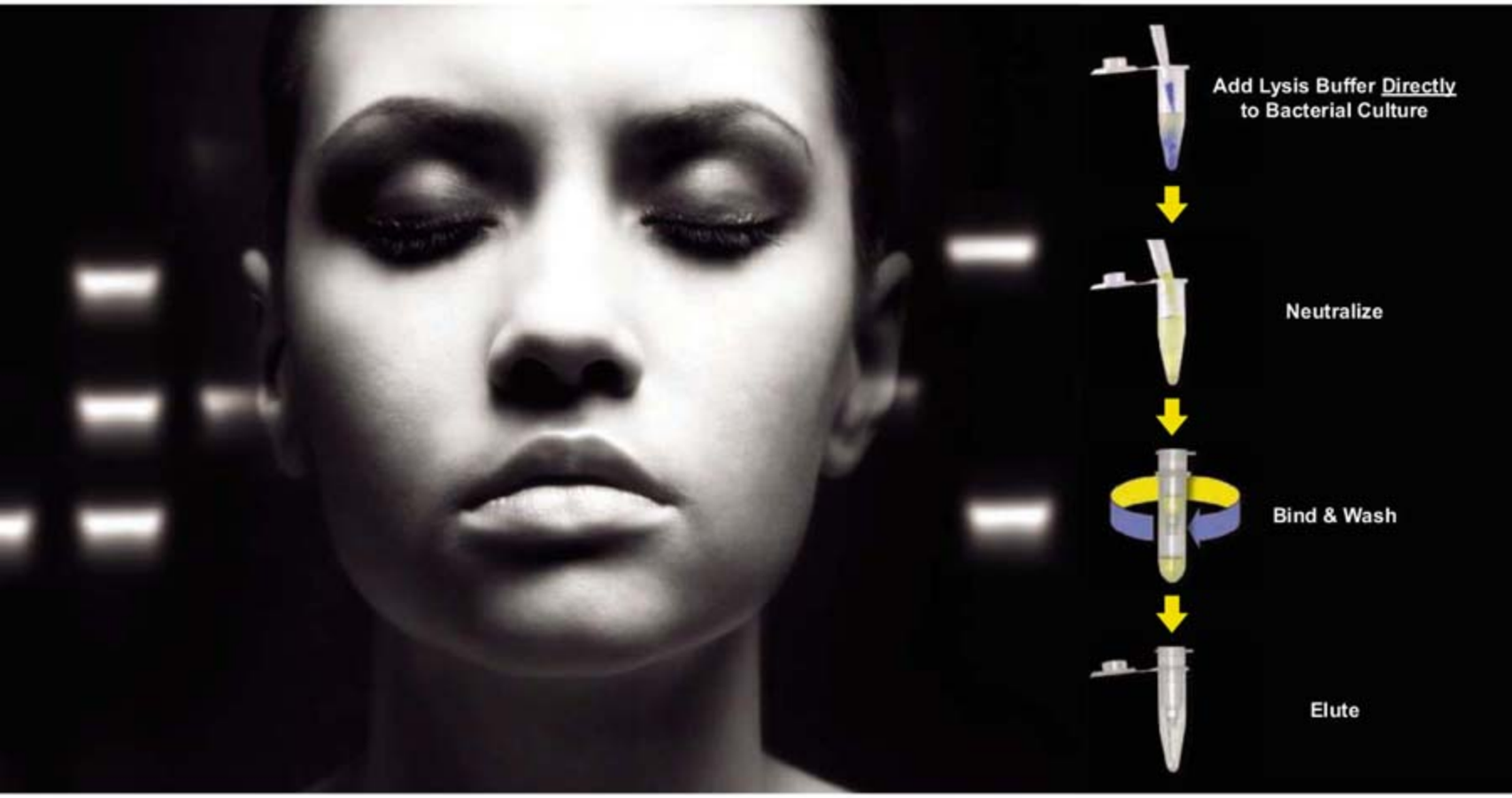

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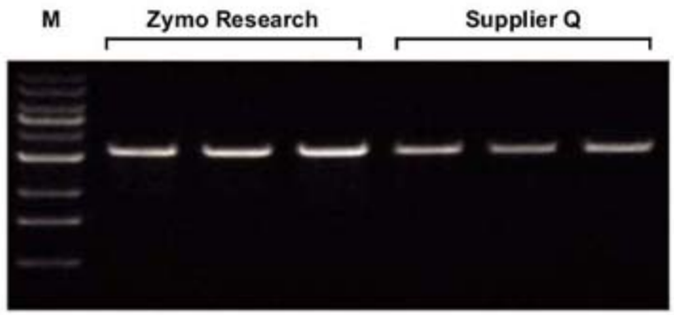

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Volume 317, Issue 5834

COVER

Universities around the world differ greatly, but the challenges facing those who teach undergraduate science, math, and engineering courses are surprisingly similar. Learn why in a special section that begins on page 63.

Photo: Getty Images

DEPARTMENTS

EDITORIAL

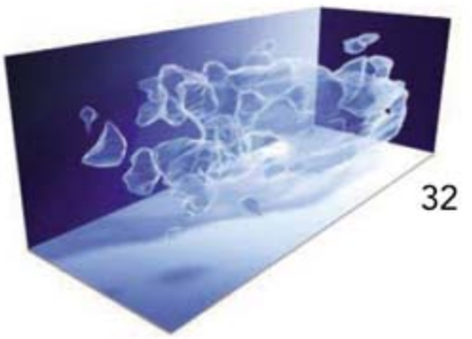
SPECIAL SECTION

The World of Undergraduate Education

INTRODUCTION

NEWS

>> Editorial page 17; for related online material, see page 11 or go to www.sciencemag.org/sciext/undergrad_education07/



NEWS OF THE WEEK

NEWS FOCUS

CONTENTS continued >>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NEUROSCIENCE

High-Speed Imaging Reveals Neurophysiological Links to Behavior in an Animal Model of Depression

R. D. Airan, L. A. Meltzer, M. Roy, Y. Gong, H. Chen, K. Deisseroth

Neural activity in the hippocampi of rats with depression-like symptoms reflects the degree of abnormal behavior, providing a clue to the brain circuits underlying depression.

10.1126/science.1144400

NEUROSCIENCE

Mosaic Organization of Neural Stem Cells in the Adult Brain

F. T. Merkle, Z. Mirzadeh, A. Alvarez-Buylla

The various types of new neurons that migrate to adult mouse olfactory cortex are each born in a different subregion of the stem cell area, the subventricular zone.

10.1126/science.1144914

CHEMISTRY

Label-Free, Single-Molecule Detection with Optical Microcavities

A. M. Armani, R. P. Kulkarni, S. E. Fraser, R. C. Flagan, K. J. Vahala

Shifts in the resonance frequency of a microcavity sensor functionalized with receptor molecules can detect the binding of a single molecule.

10.1126/science.1145002

CLIMATE CHANGE

Orbital and Millennial Antarctic Climate Variability Over the Past 800,000 Years

J. Jouzel et al.

A deuterium isotope record from an Antarctic ice core shows that during some interglacials, temperatures there were up to 4.5°C warmer than they have been during the Holocene.

10.1126/science.1141038

LETTERS

BOOKS *ET AL.*

POLICY FORUM

PERSPECTIVES

TECHNICAL COMMENT ABSTRACTS

NEUROSCIENCE

REVIEW

ECOLOGY

BREVIA

PSYCHOLOGY

RESEARCH ARTICLES

APPLIED PHYSICS

GENETICS

CONTENTS continued >>

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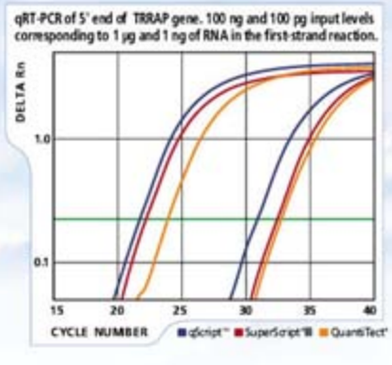

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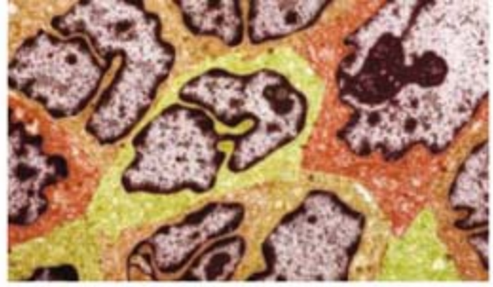
REPORTS



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CONTENTS continued >>

SPECIAL SECTION

The World of Undergraduate Education

SCIENCE'S STKE

www.stke.org SIGNAL TRANSDUCTION KNOWLEDGE ENVIRONMENT

EDITORIAL GUIDE: STNG—Signal Transduction, the Next Generation
E. M Adler and N. R. Gough
STKE supports teachers and students to foster science education.

TEACHING RESOURCE: A Biochemical Assay for Acetylcholinesterase Activity in PC12 Cells
P. J. Schwartz, J. A. Blundon, E. M. Adler
Students use a spectrophotometric assay to assess neuronal differentiation.

TEACHING RESOURCE: Instructing Graduate Students to Tackle the "Real World"—A Course Description
R. L. Patterson
Learn how one instructor designed a course intended to prepare students for a future in scientific research.

TEACHING RESOURCE: Introduction to Enzyme Kinetics—Assay of β-Galactosidase
J. K. Tillotson
Use this online tutorial to teach the basics of enzyme kinetics.

JOURNAL CLUB: Ceramide—From Embryos to Tumors
I. A. Savtchouk, F. J. Mattie, A. A. Ollis
A better understanding of the role of ceramides in apoptosis may lead to new antineoplastic therapies.

SCIENCE CAREERS

www.sciencecareers.org CAREER RESOURCES FOR SCIENTISTS

SPECIAL FEATURE: *Science* Careers for Undergrads
J. Austin
Science Careers is extending its coverage to issues and decisions facing undergraduates.

US: The Importance of Undergraduate Research
S. Webb
Prior research experience is often required by top-tier graduate schools.

EUROPE: Going Abroad for Your Ph.D.
H. Marshall
A Ph.D. abroad offers many advantages for aspiring scientists from Europe, and some obstacles.

GRANTSNET: Funding for Undergraduate Science Majors
A. Kotok and J. Fernandez
Science Careers and GrantsNet help find your next science scholarship, internship, or research opportunity.

SCIENCENOW

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Pocket-Sized Powerhouse
A neutron star infringes on the province of black holes.

Oregon Cougars to Be Hounded
New law may lead to more, not fewer, complaints about problem animals.

A Fertile Domestication of Cats
Study pinpoints the Near East as the cradle of cat domestication.

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GrantsNet Staff
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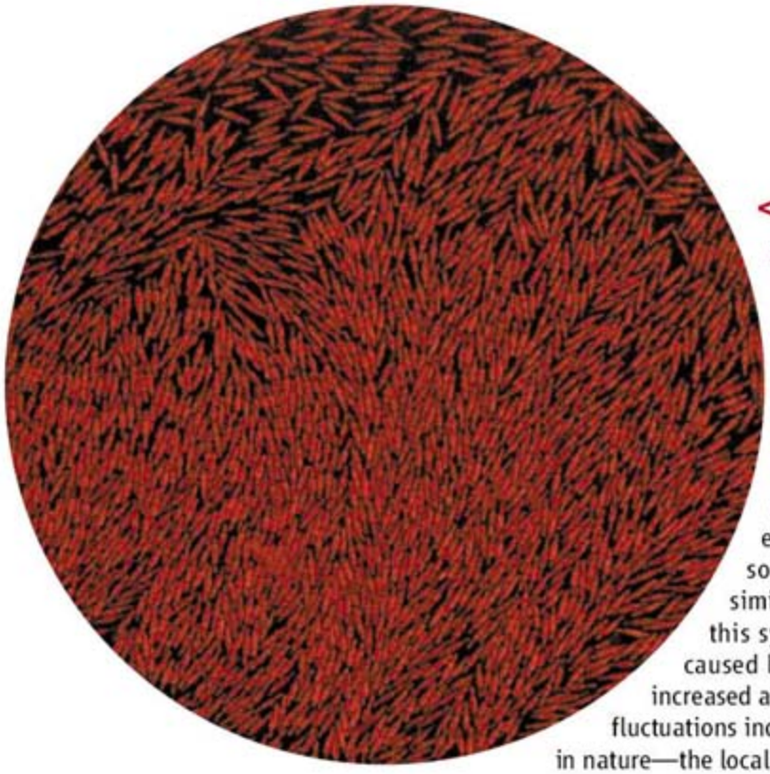

<< Small Rods, Giant Fluctuations

In nature, large-scale ordering can occur that seems to be triggered by local motions or interactions, such as in the motion of flocks of birds. On a much smaller scale, long rod-shaped molecules in solution can form a nematic liquid crystalline phase, in which the rod orientations are not isotropic but tend to align parallel to one another. **Narayan *et al.*** (p. 105; see the Perspective by **Van Hecke**) studied copper rods (about 5 millimeters in length and 0.8 millimeters in diameter, and whose ends were thinned by etching) that were confined to two dimensions and agitated so that they behaved like a fluid. The ordering behavior was similar to that of nematic liquid crystals but occurred despite this system being far from equilibrium—density fluctuations caused by changes in ordering (swarming and flocking motions) increased as particle number N, unlike the equilibrium situation where fluctuations increase as $N^{1/2}$. These persistent fluctuations are thus "giant" in nature—the local density does not reflect the overall system density.

Wireless Power Transfer

Entanglement not only applies to quantum states but also to the myriad of cords and cables that help recharge our laptop, cell phones, and other portable devices. **Kurs *et al.*** (p. 83, published online 7 June; see the Perspective by **Stewart**) report a proof-of-principle demonstration of transferring electrical power wirelessly. Using near-field magnetic resonance between two strongly coupled induction coils, they can transfer 60 watts of electrical power with 40% efficiency across a distance of 2 meters. Because the external fields of this transmission process are mainly magnetic in character, the health risks should be less than that associated with systems that emit electrical fields.

Cold but Quick

Chemical reactions in solution generally accelerate with rising temperature, but recent studies have revealed a class of gas-phase reactions between small, neutral molecules that follow the opposite trend. This phenomenon of rapid reactivity at low temperature bears on our understanding of the chemical reactions that may occur in cold interstellar clouds, which are challenging to probe experimentally. **Sabbah *et al.*** (p. 102) have performed precise laboratory rate measurements of O atom reactions with gas-phase alkenes between ~20 to 300 kelvin. They then modeled the unusually rapid low temperature rates using a theoretical framework that includes two transition states, one of which involves low-energy rearrangement of a transiently stable pre-reaction complex. The results show promise for extensions of the method to other reaction systems of astrochemical interest.

Nailing the Myxozoa

The Myxozoa, which are primarily unicellular parasites, have defied phylogenetic placement for many years and have alternatively been classified as members of the protist or animal kingdoms. **Jiménez-Guri *et al.*** (p. 116) have performed a phylogenetic analysis of an amino-acid alignment and find that the myxozoan *Buddenbrockia plumatellae*—a strange worm discovered more than a century ago—is actually an active, muscular, writhing, worm-shaped cnidarian. The existence of a worm within the Cnidaria, which includes jellyfish and corals, challenges views on body-plan evolution.

Evidence from a Greener Greenland

At present, glaciers cover about 10% of Earth's terrestrial surface, but there is only limited knowledge about the biota that occupied these vast areas before the ice formed; most fossil evidence is either deeply hidden or has been scoured away during periods of glacial expansion. **Willerslev *et al.*** (p. 111; see the news story by **Curry**) were able to extract and amplify ancient DNA reproducibly from plants and insect remains from the silty sections of deep ice cores from just above the bedrock. At the time when this ice formed, southern Greenland was covered by a diverse boreal forest consisting of pine, spruce, alder, and yew and inhabited by insects such as butterflies and moths. These results could be indicative of either extensive deglaciation of southern Greenland during the last interglacial (Eemian) or DNA survival over longer time scales of up to 1 million years.

Tools in the Toba Ash Tuff

The volcanic eruption at Toba, Indonesia, 77,000 years ago was one of the largest in Earth's recent past. This eruption likely caused dramatic cooling of Earth's climate and perhaps influenced human evolution—specifically early humans in eastern Asia—but evidence for evaluating these effects has been sparse. **Petraglia *et al.*** (p. 114) have identified the Toba ash in an archaeological sequence in India and found it to be rich with stone artifacts. The tools show a slight evolution across the ash layer but are fairly continuous. This record implies that local populations likely remained in the region and that the sophistication of the tools suggests that modern humans may have reached India by the time of the Toba eruption.

Continued on page 15

CREDITS (TOP TO BOTTOM): V. NARAYAN, S. RAMASWAMY, AND N. MENON; JIMÉNEZ-GURI *ET AL.*

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Continued from page 13

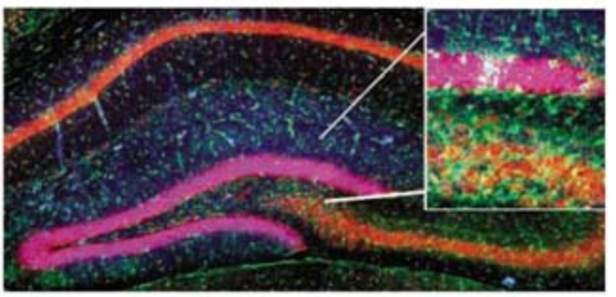
Diversity, Stability, and Controversy

The relation between diversity and stability is one of the most contentious issues in ecology: Different theories contradict each other, empirical results are inconsistent, and theoreticians and empiricists often disagree. **Ives and Carpenter** (p. 58) review this debate and point out the numerous types of stability that describe different properties of ecosystems and correspondingly numerous relations between diversity and stability. Empirical studies, however, have emphasized only a few of these relations, often ignoring those that are most important for pressing environmental concerns. Both the scope and focus of these studies should broaden to identify mechanisms that reveal generalities in diversity-stability relations.

Sea Anemone in the Spotlight

The starlet sea anemone *Nematostella vectensis* is an emerging cnidarian model. Despite the apparent morphological simplicity of sea anemones, jellyfish, corals, and other cnidarians, **Putnam *et al.*** (p. 86; see the news story by **Pennisi**) report considerable complexity in the genome of the sea anemone. The *Nematostella* genome establishes the antiquity of many genes that were previously thought to be unique to vertebrates and provides a different perspective on the origins of novel genes in animals.

Remembering the Fine Details



Pattern separation is the process by which two similar input representations are transformed into more dissimilar representations in order to reduce interference between the two patterns when they are subsequently stored in memory. A long-held but largely untested hypothesis is that the hippocampal dentate gyrus is involved in pattern separation. **McHugh *et al.*** (p. 94, published online 7 June; see the Perspective by **Bannermann and Sprengel**) generated a mouse line that specifically lacks *N*-methyl-D-aspartate receptors in dentate granule cells. Standard contextual fear conditioning was not affected, but the mice were unable to discriminate between two similar conditioning chambers.

Inflammation and Tumor Progression

Hepatocellular carcinoma, a common and deadly cancer of the liver, is 3 to 5 times more likely to occur in men than in women (see the Perspective by **Lawrence *et al.***). Working in a mouse model in which liver cancer is induced by exposure to a chemical carcinogen, **Naugler *et al.*** (p. 121) propose a molecular basis for this phenomenon explained by the action of the female hormone estrogen and its ability to inhibit inflammatory responses in the liver. Estrogen acts to inhibit secretion of interleukin-6 (IL-6) by liver macrophages known as Kupffer cells. Production of IL-6 was dependent on the signaling adaptor protein MyD88, which in turn may be activated by products of dying cells in the injured liver. **Rakoff-Nahoum and Medzhitov** (p. 124) implicate MyD88 in promoting another cancer, that of the intestine. Inflammation is known to be a risk factor for colorectal tumors. In a mouse model of intestinal tumorigenesis, mice lacking MyD88 showed inhibited growth and progression of tumors.

Bacterial Susceptibility: Whose Vault Is It?

The lung epithelia represent a major interface between the host and the outside microbial world and have evolved specific mechanisms to ensure the efficient clearance of pathogens. The importance of these processes is clearly evident in the lungs of cystic fibrosis patients, who are hypersusceptible to infection by *Pseudomonas aeruginosa* as a result of mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. **Kowalski *et al.*** (p. 130) provide evidence that a component of mysterious intracellular structures known as vaults also plays a primary role in the defense against this pathogen. After binding CFTR on epithelial cells, *P. aeruginosa* induced recruitment of major vault protein (MVP) to lipid rafts at the cell surface and the subsequent internalization of the bacteria. In mice, this MVP-dependent process was required for resistance to infection, which suggests that a similar process may be important in humans.

CREDIT: MCHUGH *ET AL.*

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Donald Kennedy is Editor-in-Chief of *Science*.

Science Teaching Roundup

HIGHER EDUCATION, NOT ONLY IN THE UNITED STATES BUT IN MANY OTHER NATIONS as well, has come in for recent criticism about the way it prepares undergraduate students for Life Afterward. For our readers, there's a two-way concern about science education. First, we are losing too many from the cohort of exceptionally able people who might go on to do graduate work and forge distinguished research careers. The second concern is about how well we instill in the others enough curiosity and basic understanding to qualify them as useful citizens of the modern world.

For the past year and a half, *Science* and our collaborators at the Howard Hughes Medical Institute (HHMI) have been giving some attention to programs and experiments in (mostly) undergraduate education, in a monthly Education Forum. In an announcement on this page (*Science*, 16 December 2005, p. 1741), HHMI President Tom Cech and I asked, rhetorically, why we couldn't do more for Kate, a mythic high school graduate who was excited about science in her high school but lost interest after the first overcrowded university lecture course in science. Of course, we hope that the Education Forum initiative, as it continues, will sow some seeds productive enough to keep the next generation of Kates engaged and excited.

Now, for the second time in a month, the News section of *Science* focuses on teaching and how to do it better. In the 1 June 2007 issue (p. 1270), *Science*'s News Focus described how three U.S. universities have taken special steps to do something for their best science-oriented undergraduates. Their purpose is not, as one might expect, to turn them into pre-Ph.D. researchers. Instead, Brigham Young University, the University of Texas, and the University of Colorado aim to make these students better prospects to fill the notoriously small pool of good high-school science teachers in the United States. A depressing joke, told in more than one state, goes: "What's the first name of our average high-school physics teacher?" The answer is "Coach." These institutions hope to change that.

In this week's issue, we go abroad to probe the situation internationally (p. 63): A stunningly imaginative teacher in Brazil who doubles as director of a science center; an 82-year-old woman in Beijing who has taught for six decades and survived the Cultural Revolution is developing course materials for a bilingual physics course—in text and CD-ROM—that will fill a gap to train engineers and physicists; an American woman who teaches Earth Science at the University of Akron, an urban comprehensive institution, where she knows she can make a difference. Every story has some encouragement about ways in which the quality of science education can be raised.

In the United States, there is a shrinking pool of potential science graduate students, so we need to look at the pool's input to see what happens in different kinds of institutions. Here's a look at colleges and universities of similar cost and selectivity, from a study begun at HHMI. For the decade 1986 through 1995, baccalaureate-only colleges were compared with research universities by measuring bachelor's degrees awarded in the previous decade with the number of Ph.D.'s produced later. Four of the top five institutions in proportional rank were liberal arts colleges. The top two, Reed and Swarthmore, nearly doubled the productivity of Harvard and Yale. Even the absolute numbers contain some surprises: Carleton graduates over this period earned more Ph.D.'s in chemistry than did those of Harvard, Yale, Stanford, or Princeton.

Had the research universities done as well as the liberal arts colleges, it would enlarge that pool of high-level scientists about which we worry so much. Why don't they? Maybe it's the intimacy of the college setting. An unpleasant possibility, though, is that undergraduates in research universities, following the exhortation to get into a lab and do "real research," sense the anxiety of graduate students and hear job-market horror stories from postdocs. Or they may observe the increasingly pressured work schedules of their faculty mentors, and the narrowed scope left for family life, and conclude that law or business school look like better alternatives. We better ask them.

– Donald Kennedy

10.1126/science.1147131

EDITED BY GILBERT CHIN AND JAKE YESTON

ECOLOGY/EVOLUTION

Fishing Induces Regime Change

The speed of change in ecosystems ranges from the imperceptible to the abrupt. Rapid, nonlinear changes (referred to as regime shifts) over time scales as short as 1 year are by their nature difficult to study and even more difficult to attribute to specific causes. Nevertheless, the accumulation of data over periods of decades can provide critical tests of mechanistic proposals.

Using time series data from fishery catches, long-term monitoring of plankton and planktivorous fish biomass, and oxygen concentration measurements over the past 50 years, Daskalov *et al.* describe two major regime shifts and several minor ones in the Black Sea ecosystem. Predatory fishes were heavily depleted in the 1960s, causing a cascade of effects down the food chain in the 1970s whereby top-down consumer control was replaced by bottom-up resource control of the system, which became dominated by planktivorous fishes. A second major shift happened in the early 1990s, when there was a population collapse of planktivorous fishes and an outburst of an alien jellyfish *Mnemiopsis leidyi*. The time series data suggest overfishing as the driver of both of these shifts, rather than pollution or the alien invasion per se. The top trophic level of predatory fish has not recovered (and seems unlikely to), although the appearance of the jellyfish *Beroe ovata*, which preys on *M. leidyi*, may promote the recovery of the next highest trophic layer of planktivorous fish. — AMS

Proc. Natl. Acad. Sci. U.S.A. **104**, 10518 (2007).

IMMUNOLOGY

The Markings of Diversity

Antibody diversity in B cells is achieved through the somatic rearrangement of variable-(diversity)-joining [V(D)J] genetic segments. Allelic exclusion ensures that only one recombined allele is expressed in a given cell, in part through the selective acquisition of epigenetic marking by demethylation of the allele that is to undergo rearrangement.

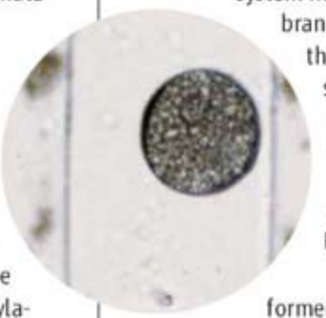
Fraenkel *et al.* show that a second major mechanism, which further enhances antibody diversity and is known as somatic hypermutation (SHM), is under the same allele-restricted control. They generated mice in which developing B cells were engineered to carry a pre-rearranged antibody kappa light chain at both alleles. In these cells, both alleles, rather than only one, were expressed, yet demethylation and extensive hypermutation were confined to just one of the two. Thus, although differences in methylation did not influence the level of transcription after recombination (explaining how both rearranged alleles could be expressed in this system), these differences did correspond to SHM levels. The findings suggest that the same epigenetic marking system that mandates monoallelic expression of productively recombined alleles also targets the rearranged antibody genes for further mutation, and that this discrimination occurs independently of transcription in mature B cells. — SJS

Nat. Immunol. **8**, 715 (2007).

CHEMISTRY

Drying and Wetting Droplets

Exploring the phase relations of complex solutions requires a convenient means of systematically varying the component concentrations. In this vein, Shim *et al.* developed a microfluidic system in which permeable membranes facilitate variation of the water composition of solute-containing droplets. Surfactant-stabilized aqueous droplets are formed in an oil stream, and the flat rectangular cross-section of the channels causes the droplets to adopt a disklike shape, so that their area changes with droplet volume. A droplet is then maneuvered into a region where the channel is connected to a reservoir via a poly(dimethylsiloxane) membrane. The reservoir can be filled either with dry air to shrink the droplet and concentrate the solute, or with water to expand the droplet and dilute the solute. This system was used to determine the aqueous phase diagram of poly(ethylene glycol) (PEG) and ammonium sulfate and to study regions of nucleation and growth of protein crystals (lysozyme) from solutions containing salts and PEG. — PDS



Droplet concentration, leading to crystallization.

J. Am. Chem. Soc. **129**, 10.1021/ja071820f (2007).

ASTROPHYSICS

Guiding the Gravity Wave Search

General relativity predicts that when massive objects crash into each other, they should emit ripples in the spacetime fabric called gravitational waves. Detection of these waves is an eagerly pursued but as yet elusive goal. The merger of binary black holes is one example of a powerful event that has been well studied theoretically in the hopes of identifying a clear gravitational wave signature. Supernovae and collapsing stars may also provide strong gravitational wave signals and thus an enlarged set of targets for detection. Dimmelmeier *et al.* performed computer simulations of fusion-burning stars progressing toward their golden years as nonburning neutron stars. The authors paid special attention to the particularly strong gravitational wave burst expected just after collapse, as the infalling material slams against the hard iron core

CREDITS (TOP TO BOTTOM): GEORGE GRALL; SHIM *ET AL.*, *J. AM. CHEM. SOC.* **129**, 10.1021/JA071820F (2007)

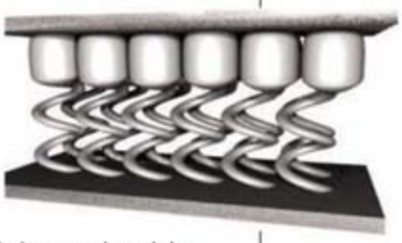
of the dying star. Exploring a wide range of parameters, they found a clear set of waveform templates that should expedite the search for gravitational waves. — DV

Phys. Rev. Lett. **98**, 251101 (2007).

APPLIED PHYSICS

Lightly Sprung

In a Fabry-Pérot interferometer, two closely spaced reflective surfaces cause multiple reflections and only partial transmission of an incoming light beam, leading to multiple interfering transmitted waves. Adjusting the distance between the mirrors finely tunes the transmitted spectrum—a useful technique in optical analysis. Dice *et al.* manufactured a nanospring-based interferometer (shown below) through glancing angle deposition of the organic material tris(8-hydroxyquinoline) aluminum (Alq_3). The springs were deposited between conducting aluminum layers that transmitted ~80% of incident light. A 6-V potential compresses the springs by 1.2 nm, shifting the peak transmission wavelength by 1.6 nm. Because Alq_3 is much softer than silicon dioxide, a material previously used for nanospring fabrication, the extensive compression does not induce breakdown of the springs. Envisioned applications include a movable mirror element in microelectrochemical systems and a pressure-sensitive optical transducer. — MSL



CREDIT: DICE ET AL., APPL. PHYS. LETT. **90**, 253101 (2007)

Appl. Phys. Lett. **90**, 253101 (2007).

CHEMISTRY

Convoluted Chromatography

A drawback of chromatographic separations is the waiting time necessary for analytes to travel from the injection site to the detector. High-throughput screens are often limited by this waiting period, during which isolated signal peaks punctuate a largely silent detection baseline. Recently, spectroscopic analysis has benefited from sophisticated mathematical algorithms that facilitate deconvolution of many overlapping signals from a single data set, thereby allowing multiple samples to be analyzed all at once. Trapp has implemented a similar multiplexing approach to gas chromatography. Specifically, he assigned a distinct binary injection sequence to each sample (with each "1" prompting injection and each "0" no action). Multiple samples were then injected continuously onto a separation column in accord with their assigned bar-code sequence, resulting in a much higher proportion of detected signals during a given time period than in traditional chromatography. The overlapping data could be deconvolved into individual chromatograms by means of a Hadamard transform and subsequent matrix manipulations. The author analyzed samples composed of several organic alcohols and hydrocarbons as a proof-of-principle and noted an enhancement in efficiency of nearly a factor of 40. — JSY

Angew. Chem. Int. Ed. **46**, 10.1002/anie.200605128 (2007).

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<< A Painful Role for Ankyrin Repeats

Transient receptor potential (TRP) channels are nonselective cation channels that sense heat and noxious chemicals, and hence are important in nociception. One family member, TRPV1, responds to capsaicin, the "hot" ingredient of chilli peppers. TRP channel activity is reduced by either desensitization (after prolonged exposure to a single stimulus) or tachyphylaxis (after sequential exposures to the same stimulus). Increased intracellular Ca^{2+} desensitizes TRPV1 currents, and this desensitization may be mediated by the calcium-binding protein calmodulin (CaM). Lishko *et al.* solved the crystal structure of the ankyrin repeat domain (ARD) found in the N terminus of TRPV1. They discovered that adenosine 5′-triphosphate (ATP), present in the crystallization solution, bound to the ARD. ATP-agarose formed a complex with purified TRPV1-ARD, which was inhibitable by free ATP. Patch-clamp assays of TRPV1-expressing cells showed that ATP sensitized TRPV1 and reduced tachyphylaxis after repeated exposure to capsaicin. Surprisingly, mutation of residues in the ATP-binding site generated mutant TRPV1 channels that had reduced tachyphylaxis, even in the absence of ATP. This suggested that another factor that promotes tachyphylaxis must bind to the same site on TRPV1, and that mutations of this site would result in a net decrease in tachyphylaxis. Exclusion chromatography analysis showed that CaM formed a complex with TRPV1-ARD that was Ca^{2+}-dependent and inhibitable by ATP. Together, these data reveal how the ARD of TRPV1 supports the sensitizing effect of ATP and the inhibitory effect of CaM. — JFF

Neuron **54**, 905 (2007).

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Officially a disease now?

More Addictions, Less Stigma

Two institutes in the National Institutes of Health (NIH) may soon get name changes to emphasize that addiction is a disease. Last week, a Senate panel agreed to change the National Institute on Drug Abuse (NIDA) to the "National Institute on Diseases of Addiction" and to rename the National Institute on Alcohol Abuse and Alcoholism (NIAAA) the "National Institute on Alcohol Disorders and Health."

The bill's sponsor, Senator Joe Biden (D–DE), said the term "abuse" is "pejorative" and doesn't convey that addiction is a brain disease. NIDA director Nora Volkow also felt that her institute's name should encompass addictions such as pornography, gambling, and food, says NIDA adviser Glen Hanson. "She would like to send the message that [we should] look at the whole field." NIAAA director Ting-Kai Li also wanted his institute's name changed to indicate that moderate drinking can be healthful.

The Senate bill—a companion to a House bill introduced by Representative Patrick Kennedy (D–RI)—was news to psychiatrist Eric Nestler of the University of Texas Southwestern Medical Center in Dallas. "My first reaction is that Joe Biden should have more important things to do," Nestler says. Expanding NIDA's purview to "diseases of addiction" seems like "overkill," he adds, given that NIH's mental health institute also funds studies on gambling and other compulsive behaviors.

Rare Bird

Wildlife researchers have taken the first photographs of one of the world's rarest birds: the recurve-billed bushbird, which lives in a dense bamboo habitat in northeastern Colombia. Paul Salaman, director of international programs at the American Bird Conservancy, says the bird uses its ultra-specialized bill to split open hollow-stemmed bamboo shoots and extract grubs and other invertebrates.


Beak customized for bamboo.

Only a few dozen bushbirds are estimated to remain. Their survival is literally miraculous: In 1709, locals spotted an image of the Virgin Mary in the root of a felled tree. The Vatican declared it a miracle, a chapel was built, and the church has protected a relict forest around it ever since. Last year, the Colombian bird preservation group ProAves declared the area a bird reserve.

Mental Illness: The Next Frontier

Developing DNA tests for schizophrenia and bipolar disorder will be the focus of a new center at Cold Spring Harbor Laboratory in New York state. The Stanley Center for Psychiatric Genomics will be established with $25 million—one of the largest gifts in the lab's 117-year history—from the Theodore and Vada Stanley Foundation.

Earlier this year, the Stanleys funded an interdisciplinary center on severe mental illnesses at the Broad Institute in Cambridge, Massachusetts (*Science*, 9 March, p. 1351). The new center has a narrower mission: "to unambiguously diagnose patients with psychiatric disorders based on their DNA sequence in 10 years' time," according to a 22 June announcement. That's a tall order, the lab's president Bruce Stillman acknowledges, because so far, only a handful of genetic variants have been strongly linked to psychiatric illnesses. The focus of the center is influenced by the fact that the Stanleys have a son with bipolar disorder, and Cold Spring's chancellor, James Watson, has a son with a "schizophrenialike" disorder, Stillman says.

The lab will use the gift to scale up its genomics efforts and hire scientists to comb DNA sequences from schizophrenia and bipolar patients for risk-related genetic variations. "I think that it is fair to say that we are witnessing a fundamental change in psychiatric genetics research," says David Porteous, a medical geneticist at the University of Edinburgh, U.K., who plans to collaborate with the new center.


Lasker with her horses.

NIH'S PATRON SAINT

She never ran a gel or trained an electron microscope on a virus, but Mary Lasker (1901–1994) had a huge impact on biomedical research. The fundraiser and lobbyist is the latest subject in the U.S. National Library of Medicine's Profiles in Science series.

NET WATCH

Lasker took illnesses personally—whether they were the frequent ear infections she suffered as a child growing up in Wisconsin or the cancer that killed her husband, Albert. "I am opposed to heart attacks and cancer and strokes the way I am opposed to sin," Lasker said. She got angry and used her connections and gift for persuasion to try to get even. One of her achievements was helping to boost the National Institutes of Health budget 150 fold in the years after World War II. >>

profiles.nlm.nih.gov/TL

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EDITED BY YUDHIJIT BHATTACHARJEE

CAMPAIGNS

ROCK ON ICE. They are in their 20s, and they have their own rock band. There is nothing unusual about that. But what's different about Matt Balmer, Tris Thorne, Ali Massey, Rob Webster, and Roger Stilwell is that they are also members of a 22-person British research team studying climate change and evolutionary biology in the frozen Antarctic. On 7 July, the group, called Nunatak (a Greenlandic word for an exposed mountain summit within an ice field or glacier), will put on a special performance at the British Antarctic Survey's Rothera Research Station, joining dozens of bands around the world in a 24-hour series of live concerts aimed at raising awareness about climate change. The event, lead by Al Gore's Alliance for Climate Protection, will be beamed to millions of viewers worldwide, and the proceeds will go toward a global effort to fight the climate crisis.

CREDITS (TOP TO BOTTOM): NATURAL ENVIRONMENT RESEARCH COUNCIL BRITISH ANTARCTIC SURVEY 2004; ARGONNE NATIONAL LABORATORY; PERIMETER INSTITUTE; JOHN-MICHAEL MAAS

MOVERS

EXOTIC FIND. It's not often that you can convince a star player on the best team in your sport to head up an expansion franchise that's still on the drawing boards. But that's essentially what the people of Argonne National Laboratory in Illinois pulled off last month, when they announced that renowned physicist Walter Henning would be rejoining the lab to lead their effort to build a new nuclear physics facility for generating rare chemical isotopes. Henning, who has worked three previous stints at Argonne, is currently the managing director for science and technology at Gesellschaft für Schwerionenforschung mbH (GSI) in Darmstadt, Germany, the world's premier facility for producing rare isotopes and smashing atoms together to create new superheavy elements.

GSI scientists have created six novel superheavy elements in recent years. But Henning and others are betting that Argonne may have the inside track on the future of rare isotope research. The lab is proposing to build a $550 million "exotic beam facility" that, with the help of new accelerator technology, is expected to markedly increase the rate at which novel isotopes can be generated and studied. Stuart Freedman, a physicist at the University of California, Berkeley, calls snagging Henning "a major coup for Argonne."

ABRUPT EXIT. The fledgling Perimeter Institute for Theoretical Physics in Waterloo, Canada, is looking for new leadership following the sudden departure of its founding director, Howard Burton, last month. According to institute spokesperson John Matlock, Burton's contract was up for renewal, but during discussions, they "didn't come to an agreement on how to move forward."

Perimeter Institute was founded in 1999 with a $75 million donation from Mike Lazaridis, co-CEO of Research in Motion (RIM), which is the maker of the ubiquitous BlackBerry wireless e-mail device, and contributions from two other RIM executives. The Canadian and Ontario governments have since added more funding. The institute has carved out a prominent niche for itself in fields such as superstring theory, quantum gravity, and quantum information theory.

Lazaridis hired Burton, a theoretical physicist from Waterloo University, to set up the institute. Burton won plaudits for hiring a cadre of young and dynamic researchers (*Science*, 5 December 2003, p. 1650). "Burton helped build the beginnings of an excellent institute," says Princeton cosmologist Paul Steinhardt, chair of Perimeter's Scientific Advisory Committee. Steinhardt's committee and senior staff are now scouting for candidates for a new director. Matlock says it's a pretty mature institute. "It's more than a startup now, so I wouldn't be surprised if there was a different tone of leadership," he says. Burton, who has written a history of the institute to be published next year, is planning a year away from science projects in southern France, where he'll be working on publishing projects.

Nonprofit World

IN GOOD HEALTH. The first director of the Global Alliance for TB Drug Development, also known as TB Alliance, a nonprofit in New York City that develops tuberculosis drugs, is leaving after 6 years to look for a new challenge. Maria Freire, who became TB Alliance's CEO and president after leading the National Institutes of Health's Office of Technology Transfer, announced her departure last month.

Freire helped oversee the growth of the organization from three staffers to 30, built a portfolio of drug candidates, and raised more than $200 million from the Bill and Melinda Gates Foundation and the governments of several countries. "It's the right time to leave, when an organization is strong," she says. Freire, who says she deliberately resigned before job-hunting to avoid "secret meetings behind closed doors," will stay on as long as a year, she says, while a search committee finds the next director. Meanwhile, she's looking for "the next challenge that will capture [her] imagination," possibly in women's or children's health.

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Priorities for astroparticle physics 29

Overhaul for science in Egypt 30

GOVERNMENT ETHICS

Supersized Lab Draws Fire at NIH's Environmental Institute

The director of the National Institutes of Health's (NIH's) environmental health institute has landed in hot water over the management of his personal lab. David Schwartz, director of the National Institute of Environmental Health Sciences (NIEHS) since May 2005, broke ethics rules, according to memos obtained by Congress, when he brought in "guest researchers" from his former employer, Duke University. The problem, along with overspending, led NIH to take the highly unusual step this spring of barring Schwartz from his own lab for about 3 months and sending about a dozen researchers back to Duke. The case raises questions about what limits NIH should apply to labs run by high-ranking officials.

The "de-Duking" process, as one NIH official described it in an e-mail, is one of several issues involving the NIEHS chief brought to light by Senator Charles Grassley (R–IA) last week. The senator also took Schwartz to task for earning about $150,000 as an expert witness in asbestos lawsuits while he was NIEHS director, despite advice from NIH ethics officials that he drop this work. These and lesser ethics problems are detailed in an 8-page, 21 June letter from Grassley to NIH director Elias Zerhouni.

Schwartz and NIH officials say misunderstandings underlie many of these problems. "I think it's clear that Dr. Schwartz did not understand the rules," says NIH deputy director Raynard Kington, referring to the lab staffing.

Schwartz, a pulmonologist specializing in environmental lung diseases, is known for discovering the role genetic variation plays in responses to inhaled endotoxins. At Duke's medical center, he was head of a department, had six research grants, and ran a lab with more than 30 people, he says. The terms of his appointment as chief of the $642 million NIEHS included a lab with 16 staff members. Because he retained a faculty position at Duke, he also agreed to recuse himself from matters involving the university.

Negotiations for the transfer of the Schwartz lab to NIH followed the usual process for incoming directors, says Michael Gottesman, NIH deputy director of intramural research. The new lab was placed under the authority of another institute to provide independent oversight. Several directors now have labs, from "very small to moderate size" of a dozen people or so, Gottesman says (see table, below).

Although Schwartz's lab fell scientifically within the National Heart, Lung and Blood Institute (NHLBI), it was administered by NIEHS because NIEHS is in North Carolina, far from NIH's main campus in Bethesda, Maryland. Kington says this resulted in a lack of "checks and balances" when Schwartz began asking for waivers to bring in more of his Duke staff. "I thought it was reasonable to allow them to continue to train with me," Schwartz says, adding that although his 26-member lab was "large," he felt it "was not impeding my ability to direct the institute."

But after a senior NIEHS offical raised questions, NIH concluded that not all of the guest appointments were covered by Schwartz's waivers, Kington says. Kington also learned that Schwartz had exceeded his lab budget of $1.8 million by more than $4 million, which Kington attributes to a mistaken assumption that his group would not be charged for using NIEHS core facilities. To make "a clean break," Kington says, Schwartz resigned as head of his lab in February, while NIH appointed an NHLBI staffer to administer the lab and moved all 12 or so guest researchers back to Duke.

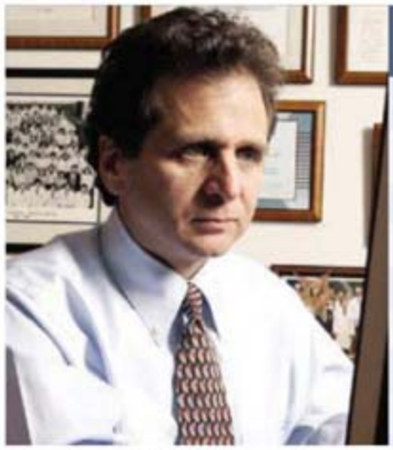
There were consequences for the guests, some of whom had been at NIEHS as long as 18 months, Schwartz says. At least two fellows had to shut down mouse experiments, according to an NIEHS scientist who asked not to be identified. Schwartz says that "it was disruptive in lots of ways," but that the trainees have found other labs and are "progressing."

The letter from Grassley also questions Schwartz's work as an expert witness on asbestos cases for law firms. Kington says this involved clinical evaluations that Schwartz had done before he came to NIEHS. Although "many at the agency had grave concerns about the activities," they felt they "could not force" Schwartz to stop, Kington says. Schwartz has discontinued the law-firm work.

This is not Schwartz's first brush with controversy. One of his first proposals at NIEHS was to privatize the institute's journal, *Environmental Health Perspectives*. He backed off after environmental groups and many scientists protested. Two senior scientists within NIEHS offer a mixed assessment. People may find Schwartz's style aggressive, but "he's really pushing us" in a good way, one says, adding that he hopes the ethics revelations won't lead to Schwartz's departure. Meanwhile, Grassley has asked NIH for more documents—including information on similar conflicts, if any, involving other directors—by 10 July.

–JOCELYN KAISER

With reporting by Marissa Cevallos.

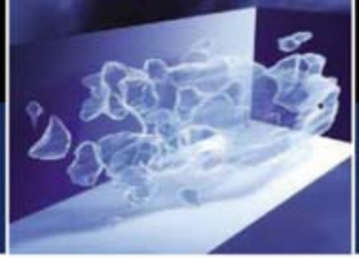


CREDIT: NIH

Balancing act. NIEHS director David Schwartz, like other NIH institute chiefs, juggles administrative duties and leads a research group. Schwartz's lab was trimmed after he violated agency guidelines.

SIZE OF SELECTED NIH DIRECTORS' LABS

Director	Institute	Scientific Staff
J. Niederhuber	NCI	9
A. Fauci	NIAID	15
E. Nabel	NHLBI	11
J. Berg	NIGMS	6
G. Rodgers	NIDDK	8
F. Collins	NHGRI	17
D. Schwartz	NIEHS	26 (now 14)



The race to shed light on dark matter
32

Ancient DNA in unlikely places
36

GENOMICS

Sea Anemone Provides a New View of Animal Evolution

Genome sequencers have just jumped down to a lower branch on the tree of life, and the view has given them a new perspective on animal evolution. The newly decoded DNA of a few-centimeter-tall sea anemone looks surprisingly similar to our own, a team led by Nicholas Putnam and Daniel Rokhsar from the U.S. Department of Energy Joint Genome Institute in Walnut Creek, California, reports on page 86. This implies that even very ancient genomes were quite complex and contained most of the genes necessary to build today's most sophisticated multicellular creatures.

"The work is truly stunning for its deep evolutionary implications," says Billie Swalla, an evolutionary developmental biologist at the University of Washington, Seattle. Until now, researchers have relied heavily on the sequenced genomes of the fruit fly, nematode, and that of a few other invertebrates to understand genome evolution leading up to the vertebrates. But the new work drives home how streamlined these invertebrate genomes have become. In contrast, the sea anemone's genome "has not changed much and retains many of the features present in our last common ancestor," says Jacek Majewski, a geneticist at McGill University in Montreal, Canada. It "seems to fill the niche essential to answer many evolutionary questions."

Animals divide into two groups, sponges and eumetazoans. The eumetazoans consist of comb jellies, cnidarians such as anemones, and bilaterians, which include everything else: limpets, lions, lobsters, and us. Comb jellies and cnidarians branched off before bilaterians diversified into the variety of animal groups known today, and they are considered relatively "simple" organisms. Cnidarians, for example, have a mouth but no anus; two tissue layers, not three; a nerve net, but no central nervous system per se.

Biologists have had plenty of bilaterian genomes to work with. But to look back in time, they needed a nonbilaterian genome for comparison—genes and genome features common to both bilaterians and nonbilaterians likely existed in their common ancestor 750 million years ago. In late 2004, Putnam, Rokhsar, and their colleagues began deciphering the 450-million-base genome of the cnidarian of choice, the starlet sea anemone, *Nematostella vectensis*.

The draft genome is already producing many surprises. Among the anemone's 18,000 or so protein-coding genes, the researchers have identified 7766 that are also present in bilaterians. Those shared genes represent the knowable part of the ancestral gene set. Three-quarters of the genes turn up in all three major animal groups examined, humans among them, but 1292 have been lost in the fruit fly and the nematode.

More than one way to do it. In addition to shedding light on evolution, the newly sequenced genome will help clarify how this sea anemone reproduces sexually, releasing eggs (*right*), and asexually, developing a second head, then cleaving across the middle of the body (*left*).

One of the big surprises of the anemone genome, says Swalla, is the discovery of blocks of DNA that have the same complement of genes as in the human genome. Individual genes may have swapped places, but often they have remained linked together despite hundreds of millions of years of evolution along separate paths, Putnam, Rokhsar, and their colleagues report. Researchers see little conservation of gene linkages in nematodes and fruit flies.

Moreover, the anemone genes look vertebratelike. They often are full of noncoding regions called introns, which are much less common in nematodes and fruit flies than in vertebrates. And more than 80% of the anemone introns are in the same places in humans, suggesting that they probably existed in the common ancestor. "The work presents a missing piece of the puzzle, which people studying intron evolution have been searching for in the past few years," says Majewski. "They present a strong validation for an intron-rich ancestor," he says.

When they compared the anemone genome with those of fungi, plants, and protists, which include slime molds and ciliates, the researchers determined that 1500—20%—of the ancestral genes originated after animals diverged from plants and fungi. Some genes appear to be completely new. Others, including ones for cell-adhesion proteins and signaling molecules, are combinations of new sequences and much more ancient DNA or combinations of parts of ancient genes. These novel genes set the stage for the evolution of highly organized tissues, notably nerves and muscles, subsequently seen in bilaterians, says co-author John Finnerty of Boston University.

Finnerty and his graduate student James Sullivan also looked in the anemone genome for 283 human genes involved in a wide range of diseases. They will report in the July issue of *Genome* that they found 226. Moreover, in a few cases, such as the breast cancer gene *BRCA2*, the anemone's version is more similar to the human's than to the fruit fly's or to the nematode's.

All these results go to show, says Finnerty, that "*Nematostella*'s genome may provide more insights into the functional evolution of human genes than many far more closely related animals."

–ELIZABETH PENNISI

CREDIT: ADAM REITZEL

CLIMATE CHANGE

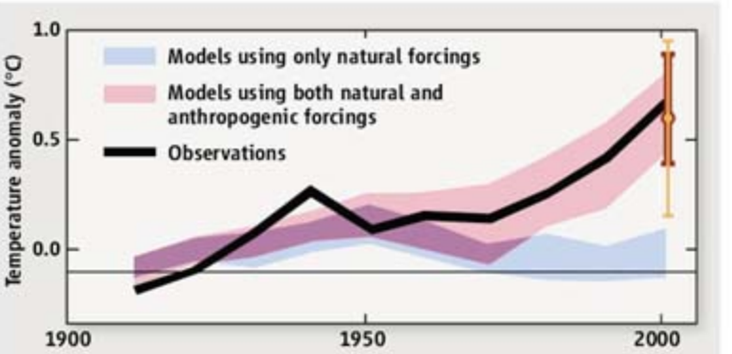
Another Global Warming Icon Comes Under Attack

Climate scientists are used to skeptics taking potshots at their favorite line of evidence for global warming. It comes with the territory. But now a group of mainstream atmospheric scientists is disputing a rising icon of global warming, and researchers are giving some ground.

The challenge to one part of the latest climate assessment by the Intergovernmental Panel on Climate Change (IPCC) "is not a question of whether the Earth is warming or whether it will continue to warm" under human influence, says atmospheric scientist Robert Charlson of the University of Washington, Seattle, one of three authors of a commentary published online last week in *Nature Reports: Climate Change*.

Instead, he and his co-authors argue that the simulation by 14 different climate models of the warming in the 20th century is not the reassuring success IPCC claims it to be. Future warming could be much worse than that modeling suggests, they say, or even more moderate. IPCC authors concede the group has a point, but they say their report—if you look in the right places—reflects the uncertainty the critics are pointing out.

Twentieth-century simulations would seem like a straightforward test of climate models. In the run-up to the IPCC climate science report released last February (*Science*, 9 February, p. 754), 14 groups ran their models under 20th-century conditions of rising greenhouse gases. As a group, the models did rather well (see figure). A narrow range of simulated warmings (purple band) falls right on the actual warming (black line) and distinctly above simulations run under conditions free of human influence (blue band).



Not so certain. The uncertainty range in the modeled warming (red bar) is only half the uncertainty range (orange) of human influences.

But the group of three atmospheric scientists—Charlson; Stephen Schwartz of the Brookhaven National Laboratory in Upton, New York; and Henning Rodhe of Stockholm University, Sweden—says the close match between models and the actual warming is deceptive. The match "conveys a lot more confidence [in the models] than can be supported in actuality," says Schwartz.

To prove their point, the commentary authors note the range of the simulated warmings, that is, the width of the purple band. The range is only half as large as they would expect it to be, they say, considering the large range of uncertainty in the factors driving climate change in the simulations. Greenhouse-gas changes are well known, they note, but not so the counteracting cooling of pollutant hazes, called aerosols. Aerosols cool the planet by reflecting away sunlight and increasing the reflectivity of clouds. Somehow, the three researchers say, modelers failed to draw on all the uncertainty inherent in aerosols so that the 20th-century simulations look more certain than they should.

Modeler Jeffrey Kiehl of the National Center for Atmospheric Research in Boulder, Colorado, reached the same conclusion by a different route. In an unpublished but widely circulated analysis, he plotted the combined effect of greenhouse gases and aerosols used in each of 11 models versus how responsive each model was to a given amount of greenhouse gases. The latter factor, called climate sensitivity, varies from model to model. He found that the more sensitive a model was, the stronger the aerosol cooling that drove the model. The net result of having greater sensitivity compensated by a greater aerosol effect was to narrow the apparent range of uncertainty, as Schwartz and his colleagues note.

"I don't want certain interests to claim that modelers are dishonest," says Kiehl. "That's not what's going on. Given the range of uncertainty, they are trying to get the best fit [to observations] with their model." That's simply a useful step toward using a model for predicting future warming. ▶

CREDIT: ADAPTED FROM S. E. SCHWARTZ *ET AL.*, *NATURE REPORTS: CLIMATE CHANGE*, 23-24 (2007)

SCIENCE POLICY

Science Gets New Home in U.K. Government

Science appears to have a more prominent role in the British government after the cabinet reshuffle that followed last week's handover of power from Prime Minister Tony Blair to his successor Gordon Brown. One of Brown's first acts was to create a new ministry whose responsibility includes both research and higher education. "The government's long-term vision [is] to make Britain one of the best places in the world for science, research, and innovation," Brown said in a statement. Researchers have cautiously welcomed the new arrangement. "The challenge for John Denham, the new minister, will now be to ensure that the department has a strong voice at the cabinet table," says cosmologist Martin Rees, president of the Royal Society.

The United Kingdom's science budget had been managed by the Department for Trade and Industry, and higher education, by the Department for Education and Skills. But their coming together in a new Department for Innovation, Universities, and Skills (DIUS) is causing concern because a single department is now responsible for both arms of the "dual support" funding system—competitive grants provided by the research councils and direct funding to university science departments. "John Denham is going to have to ensure that the two halves remain distinct and that both sustain high levels of funding," says Peter Cotgreave of the Campaign for Science and Engineering.

Alarm bells have recently been ringing over a decline in the number of students opting to study science at university (see p. 68 and *Science*, 4 February 2005, p. 668). The DIUS "will have to have strong links with [the new] Department for Children, Schools, and Families in order to ensure that young people are choosing to study science and engineering at a higher level," Cotgreave says.

And researchers have one other beef with the plan for DIUS: "We would have preferred the word 'science' to appear in the title," says Rees. –DANIEL CLERY

IPCC modelers say they never meant to suggest they have a better handle on uncertainty than they do. They don't agree on how aerosols came to narrow the apparent range of uncertainty, but they do agree that 20th-century simulations are not IPCC's best measure of uncertainty. "I'm quite pleased with how we're treating the uncertainties," says Gabriele Hegerl of Duke University in Durham, North Carolina, one of two coordinating lead authors on the relevant IPCC chapter, "but it's difficult to communicate" how they arrived at their best uncertainty estimates.

Hegerl points out that numerical and graphical error ranges in the IPCC report that are attached to the warming predicted for 2100 are more on the order expected by Schwartz and his colleagues. Those error bars are based on "a much more complete analysis of uncertainty" than the success of 20th-century simulations, she notes. It would seem, as noted previously (*Science*, 8 June, p. 1412), IPCC could improve its communication of climate science.

–RICHARD A. KERR

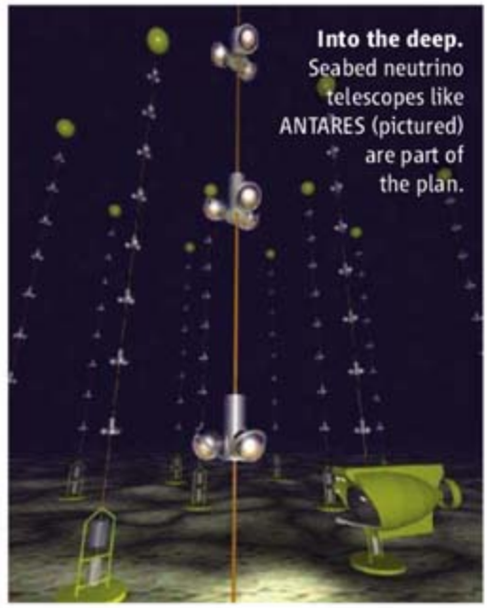
ASTROPARTICLE PHYSICS

A Road Map for European Facilities

The youthful field of astroparticle physics—the study of the universe via the cosmic rays, gamma rays, gravity waves, and neutrinos that rain down on Earth—has a growing appetite for infrastructure funding. Last week, a body representing astroparticle physicists across Europe released the first draft of a wish list of facilities. "We're trying to decide which large infrastructures can be funded in the next 10 years," says Stavros Katsanevas of France's National Institute for Nuclear and Particle Physics.

Physicists studying these high-energy visitors from space use a wide range of techniques—vast caverns filled with water to detect neutrinos, arrays of telescopes to spot the flash of light when a high-energy gamma ray hits the upper atmosphere, and interferometers with arms several kilometers long to sense gravity waves. In 2001, six European funding agencies formed the Astroparticle Physics European Coordination (ApPEC) to pool their efforts in the field. A committee was set up 3 years ago to develop a road map and this effort was joined in 2006 by a new European Union (E.U.)–funded astroparticle physics network called ASPERA.

The road map committee divided the field into seven themes, including dark-matter searches, charged cosmic-ray detectors, and neutrino experiments, and asked researchers to propose facilities. Through town meetings and dialogue with researchers, the committee came up with its highest priority projects for each theme. "We covered practically every project in Europe or with European participation," says committee chair Christian Spiering of DESY, Germany's particle physics lab. Although the committee declares that all the highest ranked projects are needed, ApPEC pushed four to the front of the line for E.U. funding: a new telescope array for gamma rays, a dark matter detector, an underground detector for neutrino astronomy and proton decay, and a next-generation gravity wave interferometer.



Into the deep. Seabed neutrino telescopes like ANTARES (pictured) are part of the plan.

CREDIT: F. MONTANET, CNRS/IN2P3 AND UJF FOR ANTARES

ASPERA coordinator Katsanevas says this sort of consensus-building exercise is essential in Europe, where there are 17 national funding agencies with interests in astroparticle physics. Working groups for each theme will now refine the draft road-map proposals with milestones and budgets and consider how they might tie in with similar efforts in the United States or Japan. At present, the total cost of the seven projects proposed (€1.2 billion) would be roughly twice the funding currently available in Europe for astroparticle physics.

European astroparticle physicists have largely welcomed the road map. "The community has been brought together more than ever before," says John Carr, spokesperson for the ANTARES Collaboration, which is constructing a neutrino telescope on the seabed off France's Mediterranean coast.

–DANIEL CLERY

Sarkozy Assumes, Bestows Control

PARIS, FRANCE—French president Nicolas Sarkozy is fulfilling a campaign promise by moving quickly to give more autonomy to his country's 85 universities. His cabinet is reviewing a bill on the topic this week expected to be debated this month in the National Assembly, where Sarkozy's UMP party has a majority. University presidents and the French Academy of Sciences have welcomed the bill, but a group of trade unions calls it "unacceptable" because they say inequality between schools will increase with the competition.

Many in France say the government controls universities too tightly (see page 69). The new bill gives universities more freedom to manage budgets, investments, and real estate, and bestows new powers on school presidents, such as more control over personnel matters. Some controversial elements of the bill—including allowing universities to select students entering the master's level, instead of admitting all applicants—were scrapped after the government negotiated with unions and student movements last week. But unions still have called on their members to protest the revised bill.

–MARTIN ENSERINK

Souring on Fake Sugar

Fearful it causes cancer, 12 U.S. environmental health experts last week asked the U.S. Food and Drug Administration (FDA) to review the potential health risks of the artificial sweetener aspartame, which appears in everything from medicines to diet sodas. A study published last month in *Environmental Health Perspectives* found somewhat more leukemias and lymphomas in male rats receiving less aspartame than the recommended maximum for humans; at higher doses, the rats had a marked increase in cancers throughout the body. Pregnant rats were fed the sweetener, and animals received it once they'd been weaned.

The work, by scientists at the European Ramazzini Foundation of Oncology and Environmental Sciences in Bologna, Italy, is "more sensitive and more realistic" than earlier aspartame studies, says James Huff of the National Institute of Environmental Health Sciences, who signed onto the FDA letter drafted by the Washington, D.C.–based watchdog group Center for Science in the Public Interest. But because the study conflicts with earlier work, FDA spokesperson Michael Herndon says that the agency finds the study unpersuasive and that "aspartame is safe." FDA's European counterpart has not responded publicly to the study.

–JENNIFER COUZIN

SCIENCE POLICY

Egypt Plans a Shakeup of Research Programs

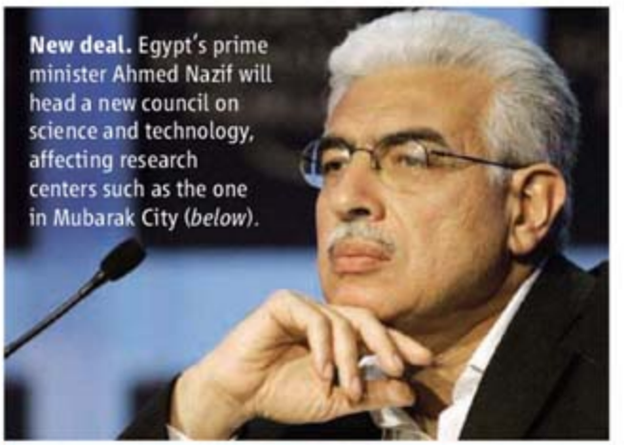
A bloated science bureaucracy and a flawed grant-awarding system have long hampered Egyptian research, with critics complaining that too little of the science budget trickles down to productive scientists. In an effort to recharge that system, Egypt's government is moving to create a research-funding agency, hike the science budget, and bolster political backing for science.

"We must have an effective mechanism for distributing research funds on a competitive basis," Egypt's science minister, engineer Hany Helal, told *Science*. The current system needs to be overhauled, he says, because innovation is lagging. At his urging, the nation's Cabinet recently approved a science restructuring plan and is awaiting a presidential decree to give it the force of law. "From the president on down, we are committed to increasing science and technology [S&T] spending and strengthening Egyptian science," Helal says.

Similar promises have been made before, but Egypt's S&T spending, as a percentage of gross domestic product (GDP) has fallen to 0.2%—well below the 1% average for developing countries. And although Egypt has the most extensive research structure in the Arab world in terms of research and development units, it ranks near the bottom among Arab countries in expenditures per scientist. Despite Egypt's traditional strengths in chemistry and engineering research, the United Nations Educational, Scientific, and Cultural Organization surveys indicate that the nation's share of the world's scientific publications has fallen over the last decade to about 0.3%, down from 0.4% in 1991, and its level of registered patents has been low. Helal says part of the new plan's goal is to jump-start innovation, for which he "wants to see more competition and more groups of researchers from different institutions or universities who apply jointly for grants."

Prime Minister Ahmed Nazif, a former computer engineering professor at Cairo University, said in a statement that he will push for Egypt to devote more of its budget to research, perhaps 10 times the current rate. He added that "restructuring the scientific research sector is one of the government's main priorities." Nazif will have an important role in the revamped system, chairing a new 18-member S&T council—modeled on a similar panel in Japan—which will include six scientists, eight Cabinet members with research portfolios, as well as representatives of industry and finance. Aly El-Shafei, a University of Cairo engineering professor who led the team that critiqued Egypt's S&T system, says "we need strong political support" to improve science. The new council will develop a plan to push S&T and increase spending, which Helal says should reach 1% of the GDP "in the short term" and would later increase beyond that.

El-Shafei says the team found "significant problems in the administration of S&T in Egypt," including excessive bureaucracy and favoritism, that could be addressed by the creation of a new funding agency with an emphasis on competitive grants. Other critics contend that too much of the science budget now goes to salaries and overhead costs at government research centers and not enough to merit-based grants.



New deal. Egypt's prime minister Ahmed Nazif will head a new council on science and technology, affecting research centers such as the one in Mubarak City (*below*).

The planned restructuring would transfer most grant-giving functions of Egypt's massive Academy of Scientific Research and Technology to the new granting agency, which will be called the Egyptian National Funding Agency. Helal says the academy "will study important topics and produce reports, but its future role will not be in funding research." The academy's acting president, agronomy professor Mohsen Shoukry—who was among the officials who took part in the restructuring talks—told *Science* that he supports the proposal for a new funding agency. He said the details of the academy's restructuring "are still under discussion."

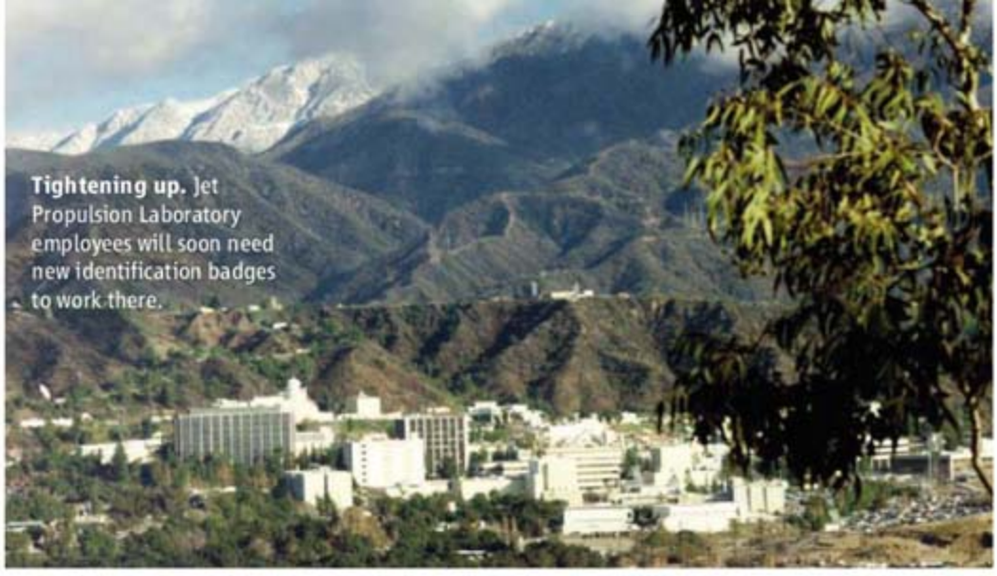
Partly because the government has not publicized the plans, Egyptian scientists are taking a wait-and-see attitude. But many would be pleased if reform means better support. "We would like to see more funding getting to the scientists who do research," says physicist Amr Shaarawi, an associate dean for research at The American University in Cairo. He says a typical Egyptian grant tends to be "very low"—a few thousand dollars. For that reason, many university-based researchers rely on international research grants from Europe, North America, and the Arab S&T Foundation.

The lone Egyptian-born science Nobel laureate, physical chemist Ahmed H. Zewail (Nobel prizewinner for chemistry, 1999) of the California Institute of Technology in Pasadena, called the Egyptian plan "a positive step forward," but added that more changes are needed. "You can't do creative science in an environment of excessive bureaucracy," he said. Zewail, who has spoken with Egypt's president about the need for science reforms, suggested several years ago that Egypt create a high-level council to promote science and also a "merit-based" funding agency, modeled in part on America's National Science Foundation.

Science minister Helal, whose portfolio includes higher education, says his ministry is also developing "a very ambitious plan for Egypt's universities" that will include numerous reforms. He is moving at the same time to expand Egypt's international scientific collaborations. Helal thinks the presidential decree that will set in motion the science restructuring could come as soon as this month.

–ROBERT KOENIG

CREDITS (TOP TO BOTTOM): SEBASTIAN DERUNGS/REUTERS; L. FRANK



Tightening up. Jet Propulsion Laboratory employees will soon need new identification badges to work there.

CREDIT: COURTESY NASA/JPL-CALTECH

HOMELAND SECURITY

NASA Lab Workers Decry New Security Checks

Aerospace engineer Dennis Byrnes prefers the open work environment at NASA's Jet Propulsion Laboratory (JPL) in Pasadena, California, to a former job with a defense contractor that required a high-level security clearance. But a new rule requiring federal contractors to undergo an extensive background check before receiving an identification badge has given Byrnes an uncomfortable sense of déjà vu. "I came to JPL to get away from the culture of secrecy," he says. "Now I feel like I'm back in it."

The new rule, which stems from a 2004 directive issued by President George W. Bush to improve security at federal facilities, requires workers to provide their fingerprints and give the government permission to collect information about their past from "schools, residential management agents, employers, criminal justice agencies, retail business establishments, or other sources of information." Federal workers have been required to do this for years; the president's directive extends the requirement to contractors working at federal facilities.

JPL is managed by the California Institute of Technology in Pasadena, but its infrastructure is owned by NASA—unlike many Department of Energy labs, which are owned by their contractors. "All of our property is federal property, and the president's directive says individuals working on federal property must undergo the same background checks that have been required of civil servants," says Veronica McGregor, a JPL spokesperson. Under that interpretation, most of the lab's 11,000 workers are affected, and NASA administrator Michael Griffin has made it clear that they have no choice. "If you do not want to surrender the information to allow your background to be checked … then you cannot work within the federal system," Griffin told JPL employees during a 4 June visit.

That message hasn't gone down well among some JPL employees. "Signing this form amounts to inviting the government to go on an open fishing expedition," says planetary scientist Robert Nelson. One employee of 39 years, technical writer Susan Foster, submitted her resignation after learning of the new policy this spring. Rumblings of protest have also arisen at NASA's Goddard Space Flight Center in Greenbelt, Maryland, which has a large number of contractors.

Nelson and three JPL colleagues have complained to two former physicists now in Congress, Representatives Vernon Ehlers (R–MI) and Rush Holt (D–NJ), that the new requirement could hurt the federal government's ability to hire the "very best scientific and engineering talent to address our nation's complex technical needs." Holt says the directive is being implemented in a way that undermines "the open and free environment" required for doing science. "There is a real possibility that this rule will discourage scientists from working with the federal government," adds an aide of Holt's. On 21 May, Holt wrote to the Commerce Department, which developed a common standard for the new identification badges, asking the agency to rethink how the directive should be implemented. Commerce has yet to respond.

JPL's McGregor says anyone who objects to the policy "should work that through the court system." Byrnes and his colleagues say they are ready to hire a lawyer and sue the government. Meanwhile, JPL officials expect every employee to have new IDs before the 27 October deadline.

–YUDHIJIT BHATTACHARJEE

SCIENCESCOPE

Blueprint for Children's Study

Researchers can now weigh in on the National Children's Study (NCS), a proposed $3 billion effort ordered by Congress. Last week, the National Institutes of Health (NIH) in Bethesda, Maryland, described how it intends to track the health of 100,000 U.S. children. The roughly 600-page research plan, developed by NIH staff and outside scientists, outlines research methods and the study's 30 hypotheses—from whether pesticides cause neurological problems to how social programs influence children's health. Officials soon will post the document online, and submit it to the National Academies for a fast-track review. A more detailed protocol must be approved by the White House before the study can begin enrollment, now set for mid-2008. NIH hasn't wanted to fund the NCS, but Congress gave it $69 million in 2007 with $110 million pending for 2008.

–JOCELYN KAISER

Spending Measure Pleases Robot Constituency

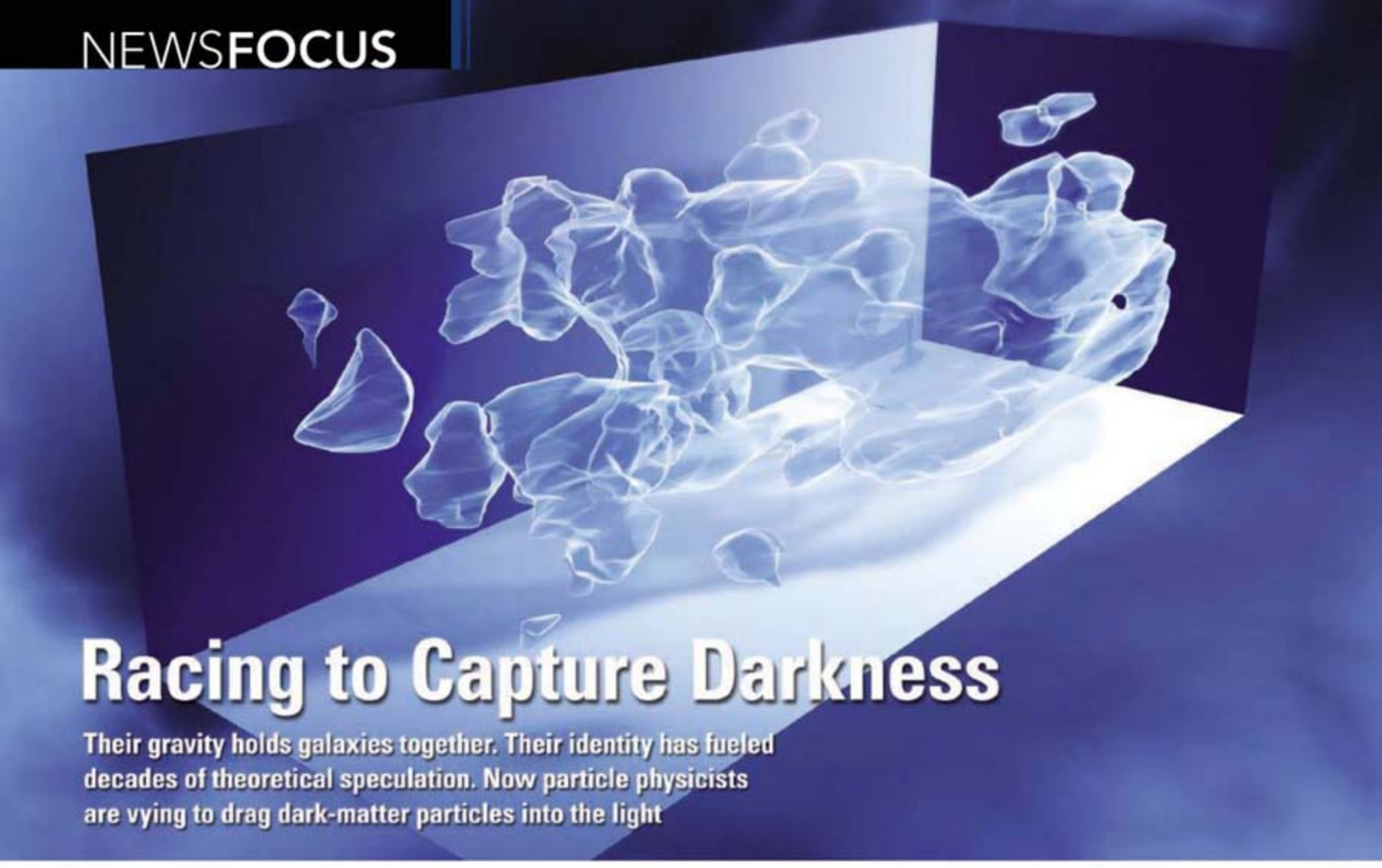
NASA earlier this year canceled plans for a series of lunar landers as precursors to the human return to the moon. Not so fast, a Senate spending panel said last week. A report accompanying a bill containing the agency's 2008 budget includes $48.7 million to keep robotic moon landers on track in the wake of a recent National Research Council report that backed such missions as scientifically valuable. Legislators also includes $2.3 million for a joint NASA-Department of Energy mission to study dark energy that the space agency wants to delay because of budget constraints, and added money for earth sciences research in line with their House counterparts. But the two bills disagree on the need to hunt for extrasolar planets. Although the House increased funding, the Senate suggests that NASA scale back its plans even more.

–ANDREW LAWLER

Ecology Lab: Not Dead Yet

Some 40 of roughly 100 staff members at the Savannah River Ecology Laboratory were let go 29 June by the University of Georgia (UGA), which manages the lab. After the Department of Energy cut $2.2 million in 2007 funding (*Science*, 18 May, p. 969), UGA failed to make up the loss. The dozen or so faculty will stay, says former director Paul Bertsch, although officials are "still trying to figure out" how to support research that is continuing with outside funding; a UGA official says "university efficiencies" will pick up much of the slack.

–ELI KINTISCH



Racing to Capture Darkness

Their gravity holds galaxies together. Their identity has fueled decades of theoretical speculation. Now particle physicists are vying to drag dark-matter particles into the light

YANGYANG, SOUTH KOREA, AND BATAVIA, ILLINOIS—Deep inside Korea's Jeombong Mountain, in a vault suffused with an eldritch red glow, a giant black cube begins to unfold. One thick, lead-lined wall filled with mineral oil, along with the box's base, inches away from the rest of the structure to reveal a smaller cube of shimmering copper. A young man steps up and pulls a chain, hand over hand, and gradually, amid the clatter of steel, the face of the copper cube rises. The rarest of coins or the relics of a saint might be accorded such sanctity, but here, in an anteroom to a tunnel delved for a hydropower station in northeastern Korea, the treasure is precious only to a particle physicist. Inside the copper cube are a dozen blocks of crystalline cesium iodide, doped with thallium and wired with electronics that will register the tiniest scintilla of light produced inside the crystals. Researchers are making a few final tweaks to their crystal array before sealing it up again and beginning an otherworldly quest.

The 15 centimeters of gamma ray–blocking lead and neutron-quenching oil in the black cube, the 10 centimeters of copper that absorb x-rays from the lead, the nitrogen piped into the copper box, the red light, and the 700 meters of rock between the chamber and the outdoors all have a singular purpose: to minimize the number of spurious flashes inside the crystals. Here at the Korea Invisible Mass Search (KIMS) experiment, researchers are hoping to be the first to spot what no one—indisputably—has seen before: particles of dark matter.

After years of preparation, physicist Kim Sun Kee of Seoul National University and his KIMS colleagues began taking data here last month with a 100-kilogram array of crystals. Each day they hope to record one or two instances of weakly interacting massive particles (WIMPs)—prime candidates for dark matter—tickling cesium and iodine nuclei in a way that liberates a flash of light. That's assuming dark particles tangle with ordinary particles as many models predict. "If they don't interact with matter, we have no hope to find them," says Kim.

The KIMS experiment is one of a few dozen experiments racing to detect dark-matter particles. Like Kim's team, groups in several countries are engaged in so-called direct searches, striving to spot the particles jostling ordinary atomic nuclei. Others are turning to the skies in indirect searches that seek signs of dark-matter particles annihilating one another in the hearts of galaxies. Meanwhile, the world's most powerful atom smasher, the Large Hadron Collider (LHC) near Geneva, Switzerland, could make dark matter as soon as it turns on next spring.

"This is the epoch in which the central theoretical predictions are finally being probed," says Blas Cabrera of Stanford University in Palo Alto, California, who for a decade has stalked dark matter as the co-spokesperson of the Cryogenic Dark Matter Search (CDMS) project. "The best guess is within reach." That prospect thrills researchers. At a recent workshop* at Fermi National Accelerator Laboratory (Fermilab) in Batavia, Illinois, more than half the 170 attendees wagered that dark-matter particles will be detected within 5 years.

Discovery is not guaranteed. The favored theoretical models suggest that experimenters should soon have dark matter in their grasp, but others predict the ghostly particles will be so elusive that researchers can never hope to snare them. It's a make-or-break situation, predicts Rocky Kolb, a cosmologist at the University of Chicago in Illinois: "Either in 5 years we will know what dark matter is, or we will never know."

The WIMP miracle

Astronomers first sensed dark matter's shadowy presence more than 70 years ago.

* The Hunt for Dark Matter: A Symposium on Collider, Direct, and Indirect Searches, 10–12 May

CREDIT: NASA, ESA AND R. MASSEY (CALIFORNIA INSTITUTE OF TECHNOLOGY)

Unseen clouds. Astronomers can infer where dark matter lies in space, but nobody knows what it is.

In 1933, Fritz Zwicky of the California Institute of Technology in Pasadena calculated that the Coma Cluster of galaxies contains too little visible matter to hold itself together. Some unseen matter must supply the extra gravity that keeps the galaxies from flying into space, he reasoned. That maverick idea gained credence about 4 decades later when astronomers found that individual galaxies also lack enough luminous matter to hold on to their stars, suggesting that each galaxy is embedded in a vast clump, or "halo," of dark matter.

Evidence continues to mount. In 2003, researchers with NASA's orbiting Wilkinson Microwave Anisotropy Probe (WMAP) measured the big bang's afterglow—the cosmic microwave background—the temperature of which varies ever so slightly across the sky (*Science*, 14 February 2003, p. 991). The pattern of hot and cold spots reveals much about how the universe evolved, and researchers found they could explain the observed pattern if the universe consists of 5% ordinary matter, 22% dark matter, and 73% weird space-stretching "dark energy," all interacting through gravity.

Researchers have never captured a speck of dark matter, however. Like a cosmic Cheshire Cat, the stuff hides in plain sight, presumably floating through our galaxy and the solar system and showing only its gravity as its grin. That coyness vexes physicists, who assume that dark matter must consist of particles. "This is the best evidence we have of new physics," says Jonathan Feng, a theorist at the University of California, Irvine. "It's simply a fact that there is dark matter, and we don't know what it is."

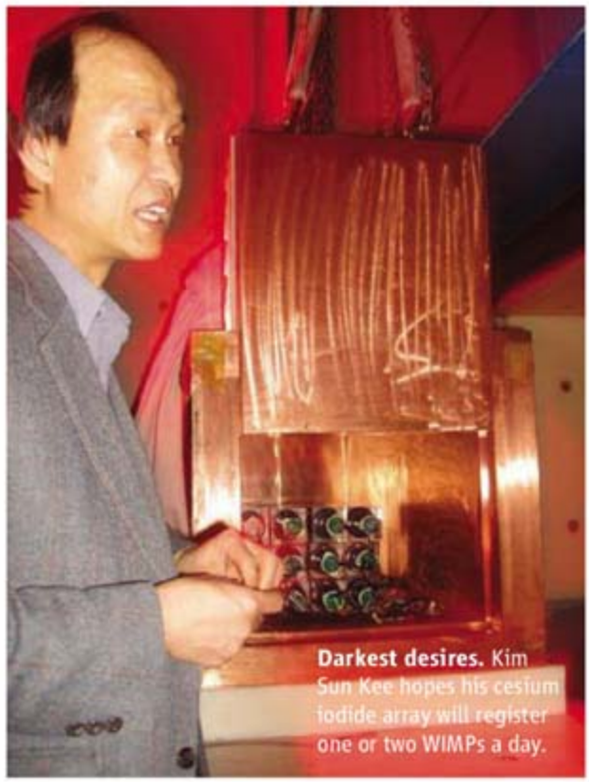
Theorists have dreamed up dozens of possibilities. Dark matter could be particles that would exist if space has minuscule extra dimensions. Or it could be particles called axions that have been hypothesized to patch a conceptual hole in the theory of the strong force that binds the nucleus.

Most promising may be the idea that dark matter consists of particles predicted by supersymmetry, a theoretical scheme that pairs every known particle with a heavier, undiscovered superpartner. The lightest superpartner, expected to be a few hundred times as massive as a proton, could be the long-sought WIMP. And if it interacts with ordinary matter as anticipated, then a simple calculation shows that roughly the right amount of WIMPy dark matter should remain from the big bang. That uncanny coincidence, or "WIMP miracle," suggests that supersymmetry is more than another stab in the dark, Feng says.

CREDIT: MUTSUMI STONE

Detecting is believing

The proof is in the particles. The most obvious way to find them is to catch them bumping into ordinary matter, and the KIMS experiment joins more than a dozen experiments that are hunting for collisions with ever greater sensitivity—including one that claimed a signal. Spotting dark matter is easier said than done, however. The particles should interact with ordinary matter even more feebly than do neutrinos, which can zip through Earth unimpeded. Researchers must also shield detectors from cosmic rays and other ordinary particles so that they may perceive the soft cries of dark particles amid the din of ordinary collisions.



Darkest desires. Kim Sun Kee hopes his cesium iodide array will register one or two WIMPs a day.

In the race to capture darkness, the frontrunner for the past few years has been an experiment called CDMS, which runs in the Soudan Mine in northern Minnesota. Its 5-kilogram "cryogenic" detector consists of stacks of germanium and silicon wafers cooled to within a fraction of a degree of absolute zero. If a WIMP crashes into a nucleus, it should knock loose several electrons and produce a tiny pulse of heat. Analyzing both the charge and heat signals, researchers can look for dark-matter particles and weed out neutrons and other red herrings.

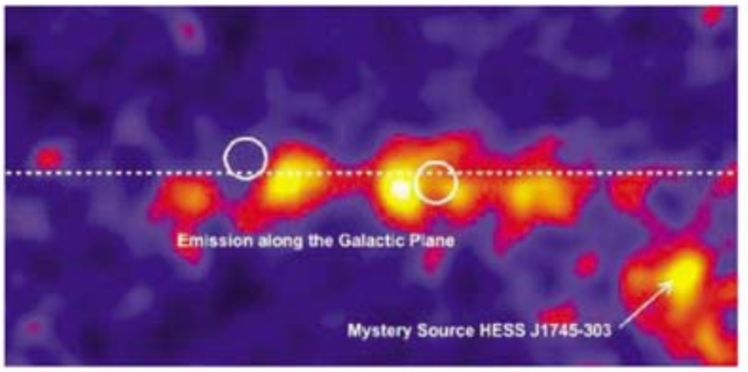
Now, another experiment has taken the lead in sensitivity. The XENON10 experiment, which resides in a tunnel in Gran Sasso, Italy, consists of a tank filled with 15 kilograms of liquid xenon. When pinged by a WIMP, a xenon nucleus should rebound through the liquid to produce a flash of light and knock free a handful of electrons. In April, the XENON10 team, led by Elena Aprile of Columbia University, reported that it had searched with five times the sensitivity of CDMS—and found nothing.

To go head to head with such efforts, the KIMS team had to start from scratch. A decade ago, Korea did not have a particle physics facility. "We always had to go abroad for research and training," says Kim, who cut his teeth at Japan's KEK accelerator laboratory in Tsukuba in the 1980s. When South Korea's science ministry launched a Creative Research Initiative in 1997, Kim, with colleagues Kim Hong Joo of Kyungpook National University in Daegu, South Korea, and Kim Yeong Duk of Sejong University in Seoul, pounced. Thrice the trio of Kims submitted their aptly named KIMS proposal, and thrice they failed. Finally, in 2000, they opted for a novel cesium iodide detector—and got funded. They caught a second break when during construction of the Yangyang Pumped Storage Power Plant, a small section off one tunnel caved in, and plant officials were amenable to hosting the experiment. "We were very lucky," says Kim Sun Kee. The collapse "opened up just enough space for the experiment."

Since then, the most arduous task has been to develop a detector largely free of trace radioactive isotopes. The KIMS team has also spent 3 years studying the scintillation signals of gamma rays and stray cosmic rays, which cause chain reactions in the atmosphere that give rise to a background "noise" of hurtling neutrons. "The neutron signal is very similar to what we expect a WIMP signal to look like," Kim explains, so the experimenters must find ways to screen it out. So far they have reduced it by 99.999%, he says.

KIMS won't immediately rival CDMS and XENON10 for overall sensitivity. But KIMS will excel in one important regard: If the WIMP-nucleus interaction depends on how each particle spins, KIMS will have a better chance of seeing the effect. "That makes KIMS complementary with CDMS and XENON10," Kim says.

KIMS can also test one of the more spectacular recent claims in physics. In 1997 and



Too bright? HESS's maps of gamma rays at the center of the Milky Way may leave clues to dark matter lost in the glare.

again in 2000, researchers with the Italian DAMA experiment at Gran Sasso reported evidence of WIMPs in a 100-kilogram array of sodium iodide crystals (*Science*, 3 March 2000, p. 1570). The team found that the rate of flashes went up and down with the seasons. That would make sense if the galaxy turns inside a cloud of WIMPs so that the solar system faces a steady WIMP wind. As Earth circles the sun, it would alternately rush into and away from the wind, causing the collision rate to rise and fall.

No other experiment has reproduced the DAMA signal, however, and most physicists dismiss the sighting. Because KIMS employs a similar detector array—with cesium iodide instead of sodium iodide—many experts say it can provide an unambiguous test of the DAMA results. DAMA group leader Rita Bernabei, a physicist at the University of Rome Tor Vergata, disagrees. "No direct comparison will be possible," she argues, because cesium iodide is less sensitive to low-mass dark-matter particles than DAMA's detectors were. In 2003, Bernabei's group fired up an upgraded 250-kilogram detector called DAMA/LIBRA. Its initial findings are due to be released next year.

The competition among dark-matter experiments is heating up. The CDMS team has already collected enough data to retake the sensitivity lead this summer. Meanwhile, researchers in North America, Europe, and Asia are deploying or planning a gaggle of ever more ambitious detectors, including XMASS, an 800-kilogram spherical liquid xenon detector that won funding this year and will be built in Kamioka, Japan. "For the first time, the direct detection experiments are moving into a regime where theorists would say that *a priori* you would expect to see something," says Lawrence Krauss, a theorist at Case Western Reserve University in Cleveland, Ohio.

Other ways to skin a cat

Meanwhile, astronomers are searching for signs of dark-matter particles in the heavens. When two WIMPs in a galactic halo collide, theory says they can annihilate each other to produce high-energy gamma ray photons or other ordinary particles. The emerging generation of gamma ray "telescopes" should be well-suited to search for such signs. Since 2004, the European-funded High Energy Stereoscopic System (HESS) in Namibia, Africa, has used its four detectors to look for light created when a gamma ray smashes into the atmosphere and triggers an avalanche of particles. Similarly, the Very Energetic Radiation Imaging Telescope Array System (VERITAS) at the base of Mount Hopkins in Arizona began taking data earlier this year. "The gamma ray observations are really the only way to measure the halo distribution and tie this all together," says James Buckley, an astronomer at Washington University in St. Louis, Missouri, who works on VERITAS.

HESS has already mapped the gamma ray glow coming from the heart of our Milky Way galaxy, the most obvious place to look for dark matter. Unfortunately, those gamma rays come overwhelmingly from more mundane sources, such as hot gas. So researchers may have to turn away from the central glare and look at so-called dwarf spheroidal galaxies that orbit our galaxy. Those galaxies should come into fuller view when NASA's Gamma-ray Large Area Space Telescope (GLAST) blasts into orbit, perhaps as early as this winter.

Dark-matter annihilations would produce other particles, too. The Russian-Italian satellite PAMELA is looking for antiprotons and other antiparticles born in the process. And IceCube, an array of 4200 light sensors being lowered into the South Pole ice, could spot neutrinos from annihilations in the sun. Zipping along with tremendous energy, measured in billions of electron volts or GeV, a few would interact with the ice to create flashes of light. A stream of 100 GeV neutrinos coming out of the sun would be a sure sign of dark matter huddling there, says Francis Halzen, a physicist at the University of Wisconsin, Madison. "How else do you get a 100 GeV neutrino out of the sun?"

Before researchers find dark-matter particles, they may be able to manufacture them. The European LHC will smash protons together at energies seven times greater than any previous collisions, recreating, in billions of tiny explosions, conditions that haven't existed since the big bang. If superpartners exist, the LHC should crank them out by the thousands, says Alex Tumanov of Rice University in Houston, Texas, who works on an LHC particle detector. "Most of these models predict that we will find or exclude the dark matter particles within 1 or 2 years," he says. "That's why everyone is so excited. We're on the doorstep."

Even if the LHC spews out new particles, however, it might not reveal enough about them to nail down which of the many versions of supersymmetry nature plays by, says Michael Schmitt of Northwestern University in Evanston, Illinois. That would require another collider that could study particles in greater detail: the proposed 40-kilometer-long International Linear Collider.

Putting it all together

Ultimately, all three methods—direct detectors, telescopes, and colliders—may have to strike pay dirt before scientists can say what dark matter is. "It's really going to require that we detect the particles in our galaxy and produce them in the lab, and that we convince ourselves that they are the same thing," says Edward Baltz, a theorist at Stanford University. In the race to spot dark matter, he says, "You don't win until everybody finishes."

Of course, the efforts may not come together so harmoniously. Direct searches might spot particles so massive that the LHC can't generate them. Or, in spite of the "WIMP miracle," dark matter might turn out to comprise several different types of particles. Researchers also face a psychological challenge if they do see something. "The first thing that you would say would be, 'Is this real?' " says Daniel Akerib, a CDMS team member from Case Western. "The first thing we would have to do is to try to make it go away" and prove it was a spurious signal, he says. That could be tricky, as it would require checking every conceivable way an ordinary particle might mimic a WIMP.

Still, that's a problem most researchers, including Kim Sun Kee, would love to have. Kim hopes that within a year, his team members will have accumulated enough data in their Korean crypt to reveal a convincing WIMP signal. The form of a WIMP behind that Cheshire grin is another question. "We don't know what a WIMP will look like," says Kim. They may soon find out—and solve one of the bigger mysteries in physics.

–ADRIAN CHO AND RICHARD STONE

CREDIT: H.E.S.S.

MICROBIAL ECOLOGY

The Dark and Mushy Side of A Frozen Continent

Researchers are uncovering a wetter world under the Antarctic ice than they ever imagined. But it's far from clear which life forms call this extreme environment home

BIG SKY, MONTANA—Wetlands might seem incongruous in Antarctica's frozen wastes. But recent expeditions have uncovered a hidden landscape of lakes, marshes, and apparent rivers sandwiched between ice and rock. These vast wetlands, imprisoned under the ice, may even be teeming with life.

"There's water everywhere under there," says John Priscu, a microbiologist at Montana State University in Bozeman. At a meeting* here last month, Priscu and other experts compared notes on the latest tantalizing clues to what this unparalleled and largely unplumbed world might be like—and laid plans for exploring it.

The first big plunge is likely to occur in Lake Vostok, the largest of Antarctica's 150-and-counting hidden lakes. A Russian-led team is preparing to penetrate and sample Vostok in 2009. The operation may help settle a point of sharp scientific dispute: whether the Connecticut-sized lake, overlain by more than 3.5 kilometers of ice, harbors microbial life. "We never thought life could exist down there," Priscu says. Now he's a believer. Other researchers are skeptics.

But experts concur that there's far more to Antarctica than meets the eye. "We're seeing a wide range of subglacial environments, from Lake Vostok to shallow, swampy environments," says Peter Doran, an earth scientist at the University of Illinois at Chicago. For now, the startling wetlands are terra incognita. Robin Bell, a geophysicist at Columbia University, says, "we've got a long way to go" before comprehending what's going on under the ice.

Peeking under the cover

The revelations about Antarctica's soggy, pitch-black underbelly have come mainly from drilling campaigns and radar mapping over the past decade. Drills that have bottomed out below the ice sheet have often hit water or warm, soft ice.

The ice blanketing the continent traps heat radiating up from Earth's core. That warmth, combined with intense pressure from the ice bearing down, allows water pockets under the sheet to keep their liquid form at normally freezing temperatures. All told, Antarctica's subglacial lakes contain around 10,000 cubic kilometers of water—about 10% of the fresh water in all the lakes elsewhere on Earth.

Water, water everywhere. An artist's rendition of aquatic Antarctica.

Antarctica's frigid water world is more dynamic than expected. Two recent studies found that some smaller subglacial lakes can roam around—they burst their banks and fill lower-elevation depressions. These findings hint at the existence of transient rivers, some as large, perhaps, as England's Thames—and raise the stakes on attempts to tap into the lakes. "We have to take a watershed approach," Doran says. If pollutants infiltrate a watershed, he says, "we may be contaminating things all the way downstream."

Although no subglacial lake has yet been pricked, researchers have drilled to within about 90 meters of Vostok's surface. Ice from this nether region is illuminating. When drilled down into from about 240 meters above the lake, the core changes from glacial ice, composed of compacted snow, to accretion ice, formed when Vostok water freezes to the ice sheet. Researchers have reported that accretion ice contains microbes that could be revived in the lab. Many scientists infer that these microbes were Vostok denizens, and other studies have shown that the microbes are close relatives of those found from Greenland to the Himalayas.

There are other signs of vitality as well. The sole sediment core under the ice sheet tested so far for microbes is brimming with life. In 2004, Brian Lanoil of the University of California, Riverside, and colleagues found that sodden soil under the Kamb Ice Stream in West Antarctica contained 10 million cells per gram—comparable to that of lake sediments found in temperate regions, and similar to sediments found under glaciers in New Zealand and Norway.

Glacial ice from the Vostok core is studded with modest numbers of microbes, around 100 cells per milliliter, according to studies led by Priscu and Brent Christner, a microbiologist at Louisiana State University in Baton Rouge. At the glacial-accretion ice transition, they reported last year in *Limnology and Oceanography*, the number rises to around 400 cells per milliliter. Accretion ice is also rich in organic carbon, Christner says. "This suggests that the lake is a source of both cells and organic carbon."

Other researchers think that the ice—and perhaps Vostok's waters—is largely sterile. Sergey Bulat, a molecular biologist at the Petersburg Nuclear Physics Institute (PNPI) in Russia, and his colleagues have also been probing the Vostok core for microbes and DNA. At the meeting, Bulat reported that his team often finds no cells in samples from both glacial and accretion ice, and never more than 20 cells per milliliter. (Bulat does put stock in one sign of life: His group has found that accretion ice contains DNA of bacteria similar to thermophilic species in vents on the ocean floor. Such microbes, he says, could be clinging to rocks around Vostok Lake and in lake sediments.)

The discrepancy between the Russian and U.S. cell counts could be due to different sampling techniques, says microbial ecologist Warwick Vincent of Laval University in Quebec, Canada. Whereas Bulat's team uses flow cytometry, Priscu and Christner count cells under a light microscope or scanning electron microscope. Or, says Vincent, "it could be that

CREDIT: ZINA DERETSKY/NSF

* Subglacial Antarctic Lake Environments, 6–8 June.

there's a lot of heterogeneity in the ice core." Others argue that Priscu and colleagues have been led astray by an artifact. To keep the Vostok borehole from freezing shut, it's filled with drilling fluid. The hydrocarbons are a feast for bacteria. Says Christner: "We can think of the borehole as a 65-ton enrichment culture."

Irina Alekhina and her colleagues at the PNPI found that some microbes in the drilling fluid match species that Christner and others have found inside cores from Vostok and from the Taylor Glacier in Antarctica—microbes that they argued were native to the ice. The primary bacteria in the drilling fluid were Sphingomonas species, known contaminants of jet fuel—like the drilling fluid, mostly kerosene. "There is no indication for indigenous microbes," Alekhina concludes.

Priscu rebuts this by pointing to a study in Antarctica's McMurdo Dry Valleys in which his group found hydrocarbon-eating microbes. "The organisms are there in nature," Priscu says. "Just because we see it in the drilling fluid doesn't mean it's not native."

That debate notwithstanding, it's a mystery how microbes can survive deep in the Vostok core, which near the bottom could be 1 million to 2 million years old. If the cells had remained frozen all that time, "their genomes would accumulate enough damage that they would effectively be dead," Christner says. One microbial refuge might be the water channels between the ice crystals, says Buford Price, a physicist at the University of California, Berkeley. Christner and biophysicist James Raymond of the University of Nevada, Las Vegas, are testing whether the microbes are specially adapted to the cold life. Raymond found that one *Chryseobacterium* species from the Vostok core produces a protein that, in the lab, blocks ice-crystal growth. This suggests the bacteria are reshaping the ice around them to minimize damage, says Christner. The protein might work as antifreeze or as a seed for crystal formation to form an ice cocoon around the bacteria.

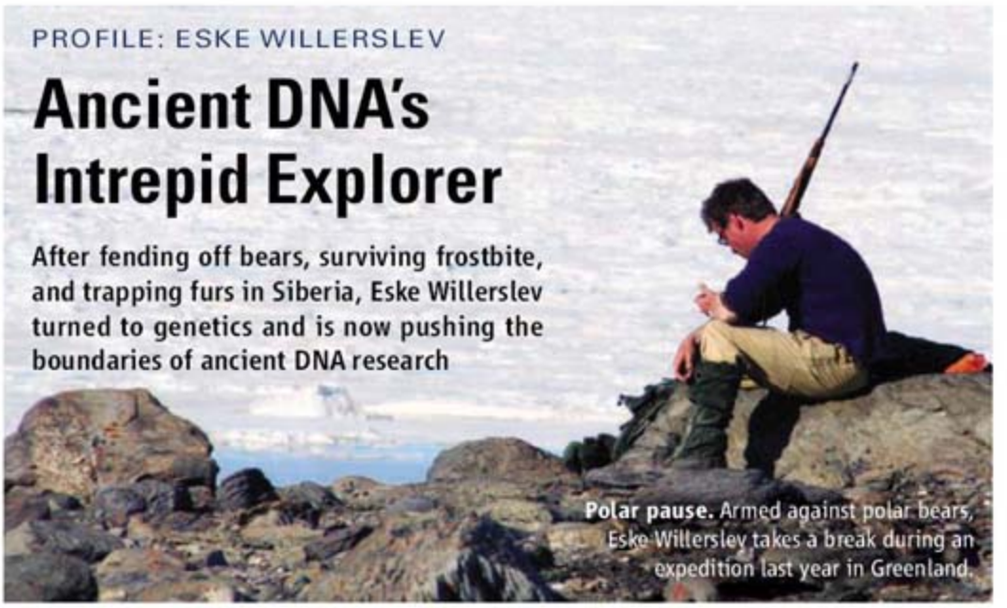
"This debate will not be resolved until Lake Vostok is sampled directly," says Vincent. When Russia breaks through, it will be like exploring a different planet. The drilling that has preceded this adventure has been "like putting pinholes in the continent," Priscu says. "We don't know what's on the bottom of that ice sheet." Well, we do know one thing: It's wet. **–MASON INMAN**

Mason Inman is a freelance journalist in Cambridge, Massachusetts.

PROFILE: ESKE WILLERSLEV

Ancient DNA's Intrepid Explorer

After fending off bears, surviving frostbite, and trapping furs in Siberia, Eske Willerslev turned to genetics and is now pushing the boundaries of ancient DNA research



Polar pause. Armed against polar bears, Eske Willerslev takes a break during an expedition last year in Greenland.

CREDIT: SVEND FULLER

COPENHAGEN, DENMARK—In the basement of the Niels Bohr Institute in Copenhagen, Jørgen Peder Steffensen pulls a puffy pale blue parka over his t-shirt and shorts and steps inside a storage locker cooled to a constant –26°C. After digging through one of the hundreds of cardboard boxes stacked inside, the bearded climatologist lifts out a dirty, plastic-wrapped cylinder of ice about 55 cm long.

The frozen chunk was cut from the bottom of an ice core drilled through Greenland's ice cap in 1981 as part of a project to look at past climate. But this core bottom was considered too disturbed by the glacier above and too contaminated with silt and dirt from below to yield much information, says Steffensen. "I've taken care of this dirty, insignificant piece of ice for 26 years," he yells as refrigeration units thunder overhead. "It was only during discussions with Eske that we homed in on a use for it."

Eske Willerslev, the director of the Centre for Ancient Genetics at the University of Copenhagen, has spent the past 8 years teasing information about the distant past from discarded ice and even less likely places. Since first extracting DNA from glacial ice in 1999, the 36-year-old biologist has pioneered what he calls "dirt DNA"—the extraction and cloning of plant and animal DNA from just a few grams of soil and ice. In 2003, he redefined ancient DNA research when he extracted the 300,000- to 400,000-year-old DNA of mammoths, bison, mosses, and much more from small samples of soil he collected from the Siberian permafrost (*Science*, 18 April 2003, p. 407). It was the oldest DNA ever discovered by more than 200,000 years.

Not long after that, Willerslev began to wonder about the ignored ice core bottoms in the building his lab shared with Steffensen's climate research group. "I did the permafrost stuff, and then suddenly it hit me: Silty ice is icy permafrost, right?" Judiciously cutting and melting the core bottoms, Willerslev and his colleagues analyzed the resulting water for signs of DNA. What Willerslev found, and reports on page 111, broke his own record for the oldest DNA ever recovered, and promises to rewrite the history of Greenland's climate. His team identified and dated genetic sequences from coniferous trees, butterflies, beetles, and a variety of other boreal forest plants—traces of ancient forests that Willerslev says covered southern Greenland perhaps as far back as 800,000 years ago.

The results have impressed his colleagues in the close-knit, highly competitive ancient DNA research community. "To go from dirty water to a forest full of insects is pretty amazing," says Matthew Collins of the University of York in the U.K. "It's spectacular how far he appears to have gone back this time."

From fur-trapping to genetics

Willerslev and his identical twin Rane grew up reading about Danish legends such as Arctic explorer Knud Rasmussen and devouring *Buddy Longway*, a popular Belgian comic book that chronicled the adventures of a fur-clad American mountain man. "I always thought I was born 200 years too late," Eske says. "Exploring America in the beginning would have fit me perfectly."

In 1991, the 19-year-old twins decided to spend their summer break in the Yakutia region of Siberia. "It was as close as you could get to unknown land," says Rane, now an anthropologist at the University of Aarhus in Denmark. "There were times when we starved and had to eat seagulls. It was very exciting at the time."

The brothers returned three summers in a row, collecting ethnographic data and filming a movie on a Siberian tribe. In 1993, a short-handed local asked Eske to spend the winter fur trapping. He readily agreed. Living like Buddy Longway "was a chance to fulfill my childhood dream," says Willerslev.

Willerslev, who spoke almost no Russian, ended up in an isolated cabin with the hard-bitten native trapper and another Russian. "We had ammunition, traps, tea, and some bread. That's it," he recalls. The team hunted moose for food, sometimes lugging home 50 kilos of meat through waist-deep snow. They were attacked by bears, and wolves picked off their hunting dogs. Willerslev once got lost alone. Only by building a fire and keeping it going all night did he manage to survive, escaping with frostbite on his face and testicles.

By Christmas, the romance of life as a trapper had completely worn off for Willerslev. But coming back to school in Denmark wasn't easy. "I was mentally changed," he says. "I tried to study for my genetics exams, but everything seemed very unimportant compared to daily survival."

Finding an ancient forest

Yet Willerslev eventually began to see opportunities that would satisfy his adventurous spirit. "I find huge satisfaction in doing exploration on a mental level instead," he says. "The 21st-century explorer is a scientist."

Interests in evolution, paleontology, and population migration soon led Willerslev to the fledgling ancient DNA field. Since no one in Copenhagen was working on ancient DNA, he improvised a self-guided course in polymerase chain reaction (PCR) techniques. He also began e-mailing with Svante Pääbo of the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, a leader in the ancient DNA field. In 2004, he traveled to Oxford to work with another pioneer Alan Cooper.

The possibilities of sequencing ancient DNA had led to an initial boom in the early 1990s. But wildly optimistic claims and *Jurassic Park*–type fishing expeditions nearly discredited the field. At issue was the tremendous vulnerability of ancient DNA techniques to contamination. PCR, the development that made ancient DNA analysis possible with its ability to copy DNA fragments in a sample many millions of times, is an indiscriminate multiplier. Any speck of DNA—from a single skin cell, say, or a single pollen grain floating in a window—would throw off an entire ancient sample with strands of modern DNA. "It's a field for which the first decade was a very faltering decade," says York's Collins. "The new generation is trained to think about nothing else but ancient DNA and contamination."

Digging deep. Eske Willerslev drills for permafrost samples in Siberia.

CREDIT: SVEND FULLER; ROSS DE MACPHEE

As part of that generation, Willerslev has combined innovative techniques with exceptionally stringent measures to control contamination. Whereas the PCR primers that latch on to DNA strands are usually aimed at just one type of organism, for his 2003 permafrost work, Willerslev used primers to grab chloroplast DNA and mitochondrial DNA from a wide variety of plants and animals. This meant he had to be particularly careful about keeping modern DNA out of reagents and permafrost samples. Tests were run in independent labs to show the results could be reproduced. Using chemicals harsh enough to break open tough microbial spores without destroying already fragmented animal DNA was another challenge—one the team solved by beating the sediments with tiny beads. "We were not only applying existing techniques to new problems," he says. "We had to combine different parts of different methods into a new protocol."

Willerslev has a reputation for being unusually intense. During a trip to Beijing, "I had to convince him to take half a day off to see the Forbidden City instead of working in a dark hotel room on papers the whole time," says Michael Hofreiter, an ancient DNA specialist at the Max Planck Institute in Leipzig, Germany. The intensity has paid off. Since returning to the University of Copenhagen in 2005—he was the youngest full professor at the university—he's built a 22-person lab from scratch.

Willerslev's ancient DNA successes have implications for a wide range of fields, from climate change to ecology. For example, glacial ice older than about 60,000 years gets too compressed by the glacier's weight and movement to provide good climate data. "It doesn't bring doubt that we have older ice, we just can't directly count it," says University of Copenhagen glacier expert Dorthe Dahl Jensen, a collaborator on Willerslev's latest research. Instead, climatologists have relied on models to argue that southern Greenland was free of ice—and open to plant growth—during the Eemian, or last interglacial period, some 130,000 to 116,000 years ago. The new results contradict that scenario: An ice-free Eemian in Greenland would have replaced the 450,000- to 800,000-year-old forest DNA Willerslev found in the bottom ice cores with younger plant and animal DNA. The survival of 450,000-year-old DNA suggests that the ice has been around much longer than previously thought. If southern Greenland remained ice-covered during the last interglacial period, it could mean global warming would have to get much worse before it completely melts away the Greenland ice sheet.

And although scientists once assumed natural degradation prevented DNA older than 100,000 years from being readable, Willerslev's ice core work opens new doors. "This means we simply don't know how far we can go back," says Hofreiter, a co-author of the new *Science* paper.

Willerslev is already eyeing Antarctica, where ice temperatures that go down to –50°C may have kept DNA preserved for even longer than Greenland's relatively balmy –20°C. "Ancient DNA hasn't peaked—in the next five years, you're going to see it going even further," he says. In a forthcoming paper in *Astrobiology*, he even asks whether ancient DNA techniques could detect traces of life on other planets. It's typical, colleagues say, of Willerslev's knack for asking unexpected questions. "While I'm doing humble domestication research, he's asking about whether there's life on Mars," says researcher Joachim Burger of the Johannes Gutenberg University in Mainz, Germany.

Willerslev's passion for the lab hasn't entirely replaced his love for the great outdoors. He is due to be married on 4 August on an island with no bridges or roads in southern Sweden. "Even the priest has to take a canoe," Willerslev says happily. "It's going to be fantastic." **–ANDREW CURRY**

Andrew Curry is a freelance journalist in Berlin.

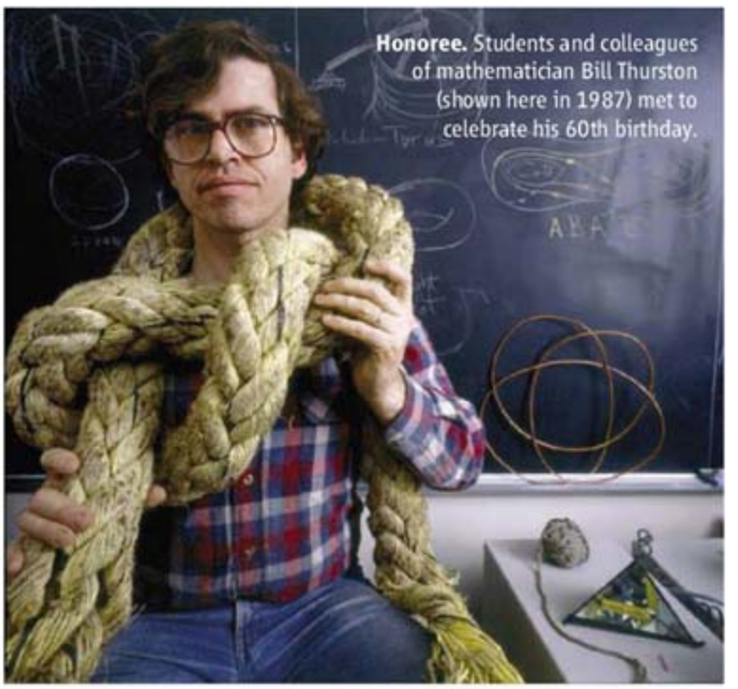
In Hyperbolic Space, Size Matters

Mathematicians since the 19th century have explored the strange realm of hyperbolic geometry, in which parallel lines behave in ways that Euclid never imagined. And cosmologists have pondered its implications for the universe ever since Einstein introduced curved space in his general theory of relativity. Modern researchers have long known that among the peculiarities of hyperbolic geometry, there is a hyperbolic three-dimensional space, or 3-manifold, of least volume. They've even long had a candidate for the smallest hyperbolic space, a tiny snarl known as the Weeks manifold. What they didn't have was proof the theory couldn't cough up something smaller yet. Now they have that too.

David Gabai of Princeton University, Robert Meyerhoff of Boston College, and Peter Milley of the University of Melbourne in Australia have shown that the Weeks manifold is indeed the smallest possible hyperbolic space. Their proof, presented here at a conference honoring the mathematician William Thurston, is part of a larger effort to understand the structure of small-volume hyperbolic 3-manifolds. Topologists would eventually like to have a list of these spaces. At least they are now sure of where to start.

The concept of least volume is meaningless in ordinary Euclidean geometry, because any shape can be scaled to any size. But the curvature of hyperbolic geometry brings with it intrinsic units of length, area, and volume. For example, you can find the area of a hyperbolic triangle by adding up its angles and subtracting the sum from π (also known as 180°). In the 1970s, Thurston, now at Cornell University, proved a surprising property of hyperbolic manifolds: Given any infinite collection of such manifolds, one member of the collection will be of smallest volume. (By contrast, for example, there is no smallest positive real number.) In particular, the entire collection of all hyperbolic manifolds must have a smallest representative.

Meyerhoff, then a graduate student of Thurston's at Princeton, found one example of a small manifold, with a volume of about 0.98136882. A few years later, Jeffrey Weeks, also a student of Thurston's, computed a smaller one, with a volume of approximately 0.94270736. "I was supposed to be working on something else," Weeks recalls. Weeks, who is now an independent geometer in Canton, New York, went on to write a program for doing computations of hyperbolic manifolds. (The program, SnapPea, is available on Weeks's Web site at www.geometrygames.org.)



Honoree. Students and colleagues of mathematician Bill Thurston (shown here in 1987) met to celebrate his 60th birthday.

The Weeks manifold is based on the space around a pair of intertwined loops known as the Whitehead link. Links and knots, which can be physically modeled by taking a tangled string of Christmas lights (or just an extension cord) and plugging its two ends together, are a fruitful source of hyperbolic manifolds. They provided some of the first evidence for Thurston's far-reaching Geometrization Conjecture (see sidebar, below); Thurston himself proved the conjecture for manifolds arising this way.

Weeks suspected that his manifold is the smallest, but he had no proof. "It was pure random chance and dumb luck," he says. The initial efforts to prove its minimality, however, went unrewarded. For a long time, the best that was known was that the smallest volume had to be at least 0.001. Only in the past 10 years did Gabai and others begin to improve the bound, first to 0.166, then to 0.33, and, just 2 years ago, to 0.67. The final nail was pounded in a paper posted to the arXiv preprint server on 30 May (arxiv.org/abs/0705.4325).

"It's pretty amazing," says Colin Adams of Williams College in Williamstown, Massachusetts. "The proof uses a huge variety of different methods," many of them brand-new, Adams notes. Gabai in particular "just doesn't quit until he gets it."

With the smallest hyperbolic manifold now known, what about the next smallest? Experts believe it's likely to be the one Meyerhoff found more than 25 years ago. But Meyerhoff says additional new ideas are needed to pin down the next manifold. Just getting the smallest volume put them right at the edge of what they could prove, he says: "We're really hanging on by our fingertips."

PRICEY PROOF KEEPS GAINING SUPPORT

No report on advances in topology is complete these days without an update on Russian mathematician Grigory Perelman's proof of Thurston's Geometrization Conjecture and its million-dollar corollary, the Poincaré conjecture (*Science*, 22 December 2006, p. 1848). After poring over Perelman's papers for 4 years, topologists are confident of the result, says John Morgan of Columbia University, who gave an overview of the proof at the Thurston conference. Much of the confidence derives from alternative proofs researchers have devised in the wake of Perelman's work. Morgan and Gang Tian of Princeton University, for example, have written a book-length exposition that "goes as far as the Poincaré conjecture" and are currently "95% of the way through the details of the Geometrization Conjecture."

"I never doubted it would be proved," Thurston said in remarks at a banquet in his honor. "It's really wonderful to see the community ownership of this mathematics."

CREDIT: ABE FRAJNDLICH

Bizarre Pool Shots Spiral to Infinity

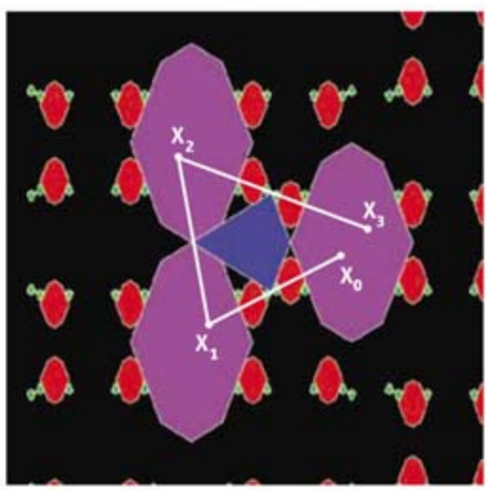
If a mathematician invites you to play billiards, watch out. You're likely to wind up trying to make shots on a table of some weird, polygonal shape—or even on the outside of such a table.

The notion of "outer billiards" was proposed in the 1950s by Bernhard Neumann and popularized (among mathematicians and mathematically minded physicists) in the 1970s by Jürgen Moser as a stripped-down "toy" model of planetary motion. The setup is simple: An object starting at a point x_0 outside some convex figure such as a polygon zips along a straight line just touching the figure to a new point x_1 at the same distance from the point of contact (see figure). It then repeats this over and over, thereby orbiting the figure in, say, a clockwise fashion. Neumann asked whether such a trajectory could be unbounded; that is, whether the object could wind up landing progressively farther and farther from the central figure. This is analogous to the question of whether planetary orbits in the solar system are stable. All proven results, however, went the other way. For regular polygons, all trajectories are bounded, and for polygons whose vertices have rational coordinates, trajectories are not only bounded but also periodic: After a finite number of steps, each trajectory winds up back where it started.

Richard Schwartz of Brown University has given a positive answer to Neumann's question: There is indeed a convex figure with an unbounded trajectory—an infinite number of them, in fact. The example turns out to involve a famous shape, the Penrose kite, which Roger Penrose introduced in the 1970s as one of two pieces (the other is known as the Penrose dart) that produce nonperiodic tilings of the plane with local fivefold symmetry.

Schwartz discovered the unbounded trajectory around the Penrose kite by writing a graphics program for systematically exploring trajectories around kites, which he picked as the simplest figures for which unbounded trajectories could possibly exist. "I think of myself as a good experimenter," he says. "I tried lots of things that didn't work out!"

A key to the discovery was that he computed not only individual trajectories but also entire regions consisting of equivalent trajectories. For the Penrose kite, he found three large, octagonal regions within which trajectories bounce periodically from one region to the other (see figure, below). Around these regions lies a cloud of smaller regions (color-coded red in figure) with similar trajectory behavior, and around these regions is a larger cloud of yet smaller regions, and so on. The larger and larger clouds of smaller and smaller regions, Schwarz found, converged to a set of points from which the trajectories are unbounded.



Outer limits. Billiard balls aimed around a Penrose kite (blue) will travel outward forever, if you pick the right starting point.

Schwartz's initial proof was heavily computational. He has made much of it conceptual, but parts are still computer-assisted. (Schwartz's program, Billiard King, is available at his Web site, www.math.brown.edu/~res.) At the same time, he has found a general class of kites for which, with the help of the computer, he can show unbounded trajectories exist. "The work is very beautiful," says Sergei Tabachnikov, a (mathematical) billiards expert at Pennsylvania State University in State College. "It is an elegant piece of programming and a deep insight into the complicated dynamical phenomena revealed by the experiments." Schwartz, however, admits that the problem is still a puzzlement: "I don't completely understand what's going on."

–BARRY CIPRA

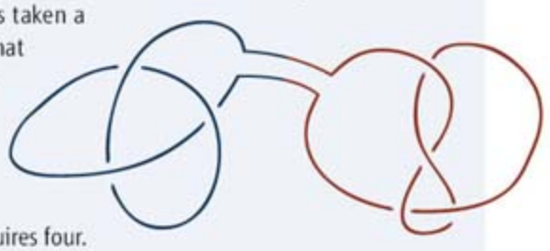
CREDITS (TOP TO BOTTOM): CHAN-HO SUH; RICK SCHWARTZ

That's Not Some Knot Sum!

Knot theory is full of simple-sounding questions that have resisted mathematicians' efforts to answer them for decades. One of the simplest has to do with the minimal number of times a knot has to cross itself when you draw it in two dimensions. In particular, if two knots are strung together to form one larger, more complicated knot (see figure), can the new knot be redrawn with fewer crossings than the original two knots combined?

"This problem has been out there forever," says knot theorist Colin Adams of Williams College in Williamstown, Massachusetts. "It's the most obvious question to ask."

Mathematicians think the answer is no, but the problem has remained stubbornly unsolved. Now, however, Marc Lackenby of Oxford University has taken a small step in the right direction. He has shown that the number of crossings cannot decrease by more than a constant factor—281, to be exact.



Knot theorists denote the minimal crossing number of a knot K by the expression c(K). The trefoil knot, for example, can be drawn with just three crossings, whereas the figure-eight knot requires four. When knots K_1 and K_2 are strung together to form a knot sum, denoted $K_1\#K_2$, the crossing number, $c(K_1\#K_2)$, is obviously no larger than $c(K_1) + c(K_2)$. The conjecture is that $c(K_1\#K_2)$ equals $c(K_1) + c(K_2)$. That is indeed true for the trefoil knot, the figure-eight knot, and all other cases knot theorists have been able to check. But the verification gets unwieldy as the number of crossings increases. It's altogether possible, Lackenby notes, that two knots, each requiring 100 crossings, could be put together and then redrawn with just 199 crossings.

Lackenby's recent result, which he began working on about a year ago, is that $c(K_1\#K_2)$ has to be at least as large as $(c(K_1) + c(K_2))/281$. The basic idea is to think of each knot as enclosed in a spherical bubble and then carefully analyze what must happen to the bubbles if the knot sum is twisted into a new shape with fewer crossings. The analysis produces the factor 281.

To prove the full conjecture, mathematicians need to whittle the number all the way down to 1. Some other approach will be needed for that effort, Lackenby says. "The number [281] is painful to work out," he notes. "One probably can reduce it further, maybe to around 100, but I'm not sure it's worth the effort."

Q AAAS

Katherine Socha, Ph.D.
I got interested in math rather late. But I liked diagramming sentences in high school. In college, I found a similar sort of architectural approach to mathematics and science.

S. James Gates Jr., Ph.D.
When I was six my father gave me some books on rockets and stars, and my universe exploded.

Leonard Susskind, Ph.D.
My father had no idea what a physicist was until I told him I wanted to be like Einstein.

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LETTERS

edited by Etta Kavanagh

A World Without Mangroves?

AT A MEETING OF WORLD MANGROVE EXPERTS HELD LAST YEAR IN Australia, it was unanimously agreed that we face the prospect of a world deprived of the services offered by mangrove ecosystems, perhaps within the next 100 years.

Mangrove forests once covered more than 200,000 km^2 of sheltered tropical and subtropical coastlines (*1*). They are disappearing worldwide by 1 to 2% per year, a rate greater than or equal to declines in adjacent coral reefs or tropical rainforests (*2*–*5*). Losses are occurring in almost every country that has mangroves, and rates continue to rise more rapidly in developing countries, where >90% of the world's mangroves are located. The veracity and detail of the UN Food and Agriculture Organization data (*2*) on which these observations are based may be arguable, but mangrove losses during the last quarter century range consistently between 35 and 86%. As mangrove areas are becoming smaller or fragmented, their long-term survival is at great risk, and essential ecosystem services may be lost.

Emerging from the embrace of a mangrove tree–lined channel in northern Brazil, these pescadores, like coastal fishers worldwide, know that healthy mangroves mean good fishing and a secure livelihood.

CREDIT: N. C. DUKE

Where mangrove forests are cleared for aquaculture, urbanization, or coastal landfill or deteriorate due to indirect effects of pollution and upstream land use (*3*, *4*), their species richness is expected to decline precipitously, because the number of mangrove plant species is directly correlated with forest size (*6*, *7*). Examples from other ecosystems have shown that species extinctions can be followed by loss in functional diversity, particularly in species-poor systems like mangroves, which have low redundancy per se (*8*). Therefore, any further decline in mangrove area is likely to be followed by accelerated functional losses. Mangroves are already critically endangered or approaching extinction in 26 out of the 120 countries having mangroves (*2*, *9*).

Deforestation of mangrove forests, which have extraordinarily high rates of primary productivity (*3*), reduces their dual capacity to be both an atmospheric CO_2 sink (*10*) and an essential source of oceanic carbon. The support that mangrove ecosystems provide for terrestrial as well as marine food webs would be lost, adversely affecting, for example, fisheries (*11*). The decline further imperils mangrove-dependent fauna with their complex habitat linkages, as well as physical benefits like the buffering of seagrass beds and coral reefs against the impacts of river-borne siltation, or protection of coastal communities from sea-level rise, storm surges, and tsunamis (*12*, *13*). Human communities living in or near mangroves would lose access to sources of essential food, fibers, timber, chemicals, and medicines (*14*).

We are greatly concerned that the full implications of mangrove loss for humankind are not fully appreciated. Growing pressures of urban and industrial developments along coastlines, combined with climate change and sea-level rise, urge the need to conserve, protect, and restore tidal wetlands (*11*, *13*). Effective governance structures, socioeconomic risk policies, and education strategies (*15*) are needed now to enable societies around the world to reverse the trend of mangrove loss and ensure that future generations enjoy the ecosystem services provided by such valuable natural ecosystems.

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16. The 2006 Australian mangrove meetings (MMM) at the Gold Coast and Daintree were sponsored by the University of Queensland, Griffith University, James Cook University, Queensland Department of Primary Industries and Fisheries, and the Ian Potter Foundation. We thank our funding sources for their support of our research on mangroves. F.D.G. is a Postdoctoral Research Scientist from the Research Foundation - Flanders (FWO-Vlaanderen). S.C.'s research is funded by the PUMPSEA project (EU 6th FP). A.M.E.'s research is supported by the Harvard Forest and by the U.S. NSF.

Supporting Undergraduate Research

THE FINDINGS OF THE EDUCATION FORUM "Benefits of undergraduate research experiences" by S. H. Russell and colleagues (27 April, p. 548) confirm the widely held belief that undergraduate research increases interest in scientific and related research careers. Indeed, as student researchers and editors with an international undergraduate journal, the *Journal of Young Investigators* (JYI; www.jyi.org), we have experienced first-hand several of the points that the authors raised.

We at JYI, however, believe that undergraduate research programs should place more emphasis on the art of scientific communication. The benefits include the opportunity to communicate undergraduate research work to a broader audience. Such an experience also develops skills necessary for the fluid but logical nature of scientific writing. These skills are otherwise missed when engrossed in wet lab work or not developed fully when merely writing final lab reports. A culture of responsibility and integrity is also developed as student authors face rigorous demands of scientific review and editing (data integrity, plagiarism, etc.).

Most importantly, the undergraduate publication experience gives students an early introduction to the world of peer review, a cornerstone of science. For JYI, a student-led journal, this benefit is doubly advantageous. Not only do student authors benefit from peer review, our JYI student reviewers are also trained in the art of reviewing, a skill not given much emphasis in undergraduate research.

JYI has been at the forefront of such undergraduate peer review and publication for 10 years since its inception in 1997. From over 500 submissions, we have published 120 undergraduate research articles. Our highlights for the past year include 10 special issues devoted to publishing research articles of various universities' Research Experiences for Undergraduates program, and participation in the recent 2007 AAAS Meeting, during which we hosted a workshop for science writing.

Our aim is to see science writing and communication play a central role in the undergraduate research experience.

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Isoprene, Cloud Droplets, and Phytoplankton

THERE IS AN ERROR THAT MAY INVALIDATE the main conclusion of the Research Article "Phytoplankton and cloudiness in the Southern Ocean" by N. Meskhidze and A. Nenes (1 Dec. 2006, p. 1419). The authors report an increase in cloud reflectivity resulting from a 30% decrease in cloud droplet effective radius and a doubling of cloud droplet number concentration over a large phytoplankton bloom in the Southern Ocean, resulting in an extra 15 W m^{-2} of energy reflected back to space. They attribute these changes to enhanced isoprene produced in the bloom. Our measurements made during the Southern Ocean Iron Experiments (SOFeX) (*1*) were used by Meskhidze and Nenes to scale seawater isoprene values based on measured chlorophyll-*a* concentrations. Unfortunately, they converted our isoprene concentrations incorrectly, resulting in a three-order-of-magnitude overestimation and hence a much greater calculated isoprene flux.

During SOFeX, we measured climate-relevant organic gases in the dynamic headspace of an equilibrator (*2*) in contact with seawater (*1*). We reported isoprene concentrations to be on average 560 pptv (parts per trillion by volume or picomoles $mole^{-1}$ of air) inside of the SOFeX North Patch (NP), which is the mixing ratio that the air above the water would have if the headspace were static. To convert from mixing ratio of static headspace to seawater concentration, we use Henry's Law:

$$C_g \times K_H = C_a \qquad \text{(Eq. 1)}$$

where C_g is the mixing ratio of a gas in equilibrium with the dissolved gas in the aqueous phase, C_a. An average Henry's law constant (K_H) for isoprene of 0.0130 M atm^{-1} was used (*3*). Therefore, the average seawater isoprene concentration in the NP was ~7.3 picomoles L^{-1} (pM). Listed in the authors' Table 2 is an average isoprene concentration of 31.4 nanomoles L^{-1} (nM) in the NP. This leads me to believe that isoprene is not the reason for their observed extra cloud albedo.

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Response

WE THANK WINGENTER FOR HIS LETTER. Indeed, we misinterpreted some of the data in Wingenter *et al.* (*1*). We were unaware that "concentration of dissolved gases measured in and around the fertilized patch" in (*1*) referred not to seawater con-

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 3 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

centrations but mixing ratios in the headspace of an equilibrator. When corrected, the values of C_w^b in Table 2 should be reduced by about three orders of magnitude (see correction on page 43). This does not, however, alter our conclusions or isoprene secondary organic aerosol (SOA) hypothesis. The fact remains that reported isoprene air-sea fluxes and concentrations in the marine boundary layer (MBL) vary by orders of magnitude, with the average concentrations between 4 and 250 pptv and fluxes of 10^7 to 6×10^9 molecules cm^{-2} s^{-1} (*2*–*6*). For the high end of measured isoprene levels in the Southern Ocean [which are attributed to enhanced phytoplankton productivity (*5*)], our simulations suggest that the amount of SOA is potentially enough to impact cloud droplet number concentrations. This large range may be from highly variable environmental conditions [i.e., photosynthetically active radiation (PAR), sea-surface temperature, wind speed, ocean mixed-layer depth, etc.] and phytoplankton speciation encountered during the experiments. Given the above and the uncertainty in isoprene-to-SOA yield, to state that "isoprene is not the reason for their observed extra cloud albedo" implies a level of understanding that currently does not exist.

We have shown a direct and strong link between phytoplankton and clouds. Given the identified potential of ocean-emitted isoprene (and other volatile organic compounds) on atmospheric oxidizing capacity and new-particle formation (*4*–*7*), the possibility of isoprene SOA contributing to the global CCN budget is real and worth exploring.

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CORRECTIONS AND CLARIFICATIONS

Perspectives: "Reassessing carbon sinks" by D. F. Baker (22 June, p. 1708). The summary sentence is incorrect. It should read, "Less carbon dioxide is taken up by the Southern Ocean, and more by tropical land areas, than previously thought."

Perspectives: "Recent progress and continuing puzzles in electrostatics" by L. B. Schein (15 June, p. 1572). The summary sentence is incorrect. It should read, "Insight into the adhesion of charged insulating particles is affecting laser printing technology and may have other industrial applications." In the figure caption, "200-μm" should read "300-μm."

Policy Forum: "Danger of deep-sea mining" by J. Halfar and R. M. Fujita (18 May, p. 987). The deep-sea mining activities were erroneously described as strip-mining operations. The correct term should be pit-mining operations, implying an absence of overburden, as stated later in the article.

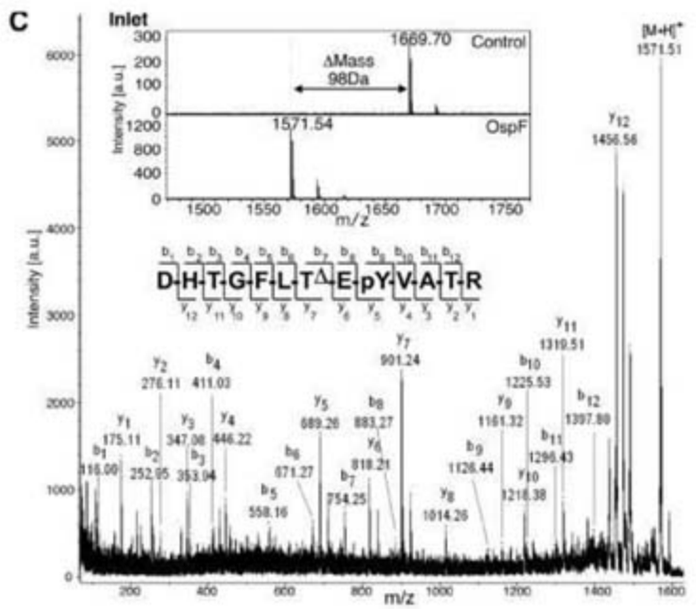
Perspectives: "A promising mimic of hydrogenase activity" by T. B. Rauchfuss (27 April, p. 553). The Perspective states that the compound discovered by S. Ogo *et al.* [*Science* **316**, 585 (2007)] catalyzes the hydrogenation of benzaldehyde to the corresponding alcohol. This statement referred to a preliminary result that was not presented in the published paper.

Table 2. Ocean chlorophyll *a*, fluxes, and atmospheric concentrations of isoprene

	[Chl *a*] (mg m^{-3})		Dissolved isoprene concentration (pM)			Isoprene flux (10^8 molecules cm^{-2} s^{-1})			Estimated MBL concentration (pg m^{-3})	
	Bloom	SOFeX	C_w^A	SOFeX	C_w^B	F_A	F_B	Amazon	Isoprene	SOA
Average	3.0	2.4	30	7.6	8.4	1.8	0.6	18200	500	20
Max	12.7	2.6	130	>10	34	8.6	2.2	20000	2000	60
Min	0.1	0.1	3.0	<2	1.4	0.2	0.1	7000	80	2

Reports: "The phosphothreonine lyase activity of a bacterial type III effector family" by H. Li *et al.* (16 Feb., p. 1000). A production error caused some of the data labels in Fig. 3C to be obscured. A corrected version appears below on the left.

Research Articles: "Phytoplankton and cloudiness in the Southern Ocean" by N. Meskhidze and A. Nenes (1 Dec. 2006, p. 1419). In Table 2, the C_w^ASOFeX, $C_w^B F_B$, isoprene, and SOA values were incorrect. The corrected table is shown above. For detailed table legend, see original paper.



TECHNICAL COMMENT ABSTRACTS

COMMENT ON "Wandering Minds: The Default Network and Stimulus-Independent Thought"

Sam J. Gilbert, Iroise Dumontheil, Jon S. Simons, Chris D. Frith, Paul W. Burgess

Mason *et al.* (Reports, 19 January 2007, p. 393) attributed activity in certain regions of the "resting" brain to the occurrence of mind-wandering. However, previous research has demonstrated the difficulty of distinguishing this type of stimulus-independent thought from stimulus-oriented thought (e.g., watchfulness). Consideration of both possibilities is required to resolve this ambiguity.

Full text at www.sciencemag.org/cgi/content/full/317/5834/43b

RESPONSE TO COMMENT ON "Wandering Minds: The Default Network and Stimulus-Independent Thought"

Malia F. Mason, Michael I. Norton, John D. Van Horn, Daniel M. Wegner, Scott T. Grafton, C. Neil Macrae

Gilbert *et al.* suggest that activity in the default network may be due to the emergence of stimulus-oriented rather than stimulus-independent thought. Although both kinds of thought likely emerge during familiar tasks, we argue—and report data suggesting—that stimulus-independent thought dominates unconstrained cognitive periods.

Full text at www.sciencemag.org/cgi/content/full/317/5834/43c

NEUROSCIENCE

The Hippocampus Review

Terrence J. Sejnowski

The Hippocampus Book—with a gestation period of 20 years, written by a team of 23 researchers, styled by 5 editors, weighing nearly 6 pounds, and running over 850 pages—summarizes 50 years of anatomical, physiological, and behavioral research on the sea horse–shaped structure buried deep within the brain's medial temporal lobe. It is, however, much more than a reference work. The book also captures the lore of the field and will serve as a reliable guide for the next generation of hippocampologists.

The volume begins with a historical perspective written by some of those responsible for the original research. Fifty years ago, Brenda Milner discovered that cutting out both hippocampi and surrounding regions from the rest of the brain (in an attempt to alleviate intractable epilepsy) had produced an almost pure case of anterograde amnesia in the patient H. M. As is usually true for individuals who cannot convert new experiences to long-term storage, H. M.'s memory of events that occurred well before the operation was spared. This was a turning point for the study of the hippocampus, which until then had been thought to be a part of the olfactory system and involved in emotions. We now know (from research on monkeys by Stuart Zola and Larry Squire) that the magnitude of the memory problem increases as more of the cortex surrounding the hippocampus is damaged; H. M. had a massive lesion of his medial temporal lobes.

The Hippocampus Book

Per Andersen, Richard Morris, David Amaral, Tim Bliss, and John O'Keefe, Eds.

Oxford University Press, New York, 2007. 868 pp. $125, £60. ISBN 9780195100273.

The chapter by Tim Bliss, Graham Collingridge, and Richard Morris could stand on its own as a 132-page monograph on hippocampal synaptic plasticity, the change in synaptic strength thought to underlie learning. Long-term potentiation (LTP), which has become an important field of research in its own right, was discovered when high-frequency stimulation of the inputs to the hippocampus resulted in more efficient subsequent responses at synapses in the dentate gyrus. LTP at hippocampal synapses is the best-studied form of synaptic plasticity in the brain, and several forms of long-term depression (in which the efficiency of synapses decreases rather than increases) were also discovered there, as was a long-term change in excitability (E-S potentiation). Although similar forms of synaptic plasticity have been reported elsewhere, the hippocampus remains the gold standard.

The limitations of the usual practice in primates of recording from single neurons while the animal is restrained in a chair are illustrated in the chapter on hippocampal physiology and behavior, in which John O'Keefe describes recordings from single hippocampal neurons in freely moving rats. If the rats had been restrained as primates are, then place cells—which respond only when a rat is near a specific place in its enclosure—would never have been discovered. When a rat enters a new environment, these cells quickly form a map of its surroundings that is stable for many days. The overall electrical activity in the hippocampus in freely moving rats oscillates at 4 to 6 Hz, which gates the activity of the place cells. The advancement of the timing of place cell spikes, relative to the phase of the 4- to 6-Hz rhythm, provides highly accurate information on the location of the rat. The implications of this observation for how precisely the brain is constructed have not yet been fully appreciated.

The hippocampus has attracted brain modelers, who have theorized the sparse distributed codes of the granule cells in the dentate gyrus as orthogonalization of its inputs and the recurrent connectivity of area CA3 as an attractor network that performs pattern completion. Neil Burgess's chapter provides an overview of these models and their consequences. The recent discovery by May-Britt and Edvard Moser of grid cells in the entorhinal cortex (cells that respond to locations on a hexagonal grid and feed into the hippocampal place cells) was not predicted by any of these theories, a missed opportunity. But the discovery was also missed by a generation of physiologists who studied rats in mazes that were too small to detect the grid pattern.

At the Society for Neuroscience meeting four years ago, Richard Morris and Susumu Tonegawa had a spirited public debate on whether we would learn more about the function of the hippocampus from studying neural circuits or from molecular genetics. In their chapter, Pavel Osten, William Wisden, and Rolf Sprengel summarize the molecular mechanisms that have been found to operate in the hippocampus and the genetic tools that have allowed specific cell types to be manipulated in different parts of the hippocampus to discover hippocampal functions, such as pattern completion in the CA3 region.

Among the most important discoveries about the brain is the finding that in adults new neurons are born in the dentate gyrus of the hippocampus and that their survival depends on experience. In an "enriched" environment (or even an exercise wheel), more

Hippocampus—Woman/Man (Tracey Shors, Steve Weiss, and Dolph Geurds, at Quark Park, Princeton, NJ, 2006).

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new granule cells survive and get wired into existing circuits. This is an encouragement to lead an active life. In her chapter on structural plasticity, Elizabeth Gould considers the possible functions of these new neurons and how stress reduces their proliferation. In contrast, antidepressants such as Prozac enhance hippocampal neurogenesis. The hippocampus continues to amaze.

The hippocampus has served as a Rosetta stone, and deciphering its secrets is helping us to understand many core principles of brain function. *The Hippocampus Book* records the impressive results of our decoding to date and passes the torch to the next generation.

10.1126/science.1142139

ANIMAL BEHAVIOR

Devoted to Dogs

Elaine A. Ostrander

With the availability of a draft assembly of the dog genome sequence (*1*), 2.5 million single-nucleotide polymorphisms, and numerous published studies on mapping complex traits (*2*–*4*), canine biologists are returning to what was for many their first love: understanding the biology of dog behavior. Toward that end, *The Behavioural Biology of Dogs*, edited by Per Jensen (an ethologist at Linköping University, Sweden), fills an important niche.

Geared to students of animal behavior and veterinarians, the book is split into four sections, each of which focuses on some aspect of dog behavioral biology. The stage is set by the first three chapters, which are devoted to understanding the evolution and domestication of the dog. Using DNA analyses, Peter Savolainen provides a clear and vivid discussion of the dog's origin, concluding that domestication is likely to have occurred only once, about 15,000 years ago, and in eastern Asia. By comparison, Carles Vilà and Jennifer Leonard address the origin of breed diversity. They offer an excellent discussion of the molecular methods used to study dog origins and then plunge into considerations of the number and timing of domestication events, arguing cogently that domestication may have occurred as much as 100,000 years earlier than suggested by Savolainen. Regardless, all agree that the wolf is the dog's closest ancestor and that behaviors observed in modern dogs originate with the wolf.

The Behavioural Biology of Dogs

Per Jensen, Ed.

CABI, Wallingford, UK, 2007. 276 pp. Paper, $70, £35, €55. ISBN 9781845931872.

Five chapters dealing specifically with basic animal behavior begin with Jensen's overview of the ethological foundation for animal behavior, which includes necessary aspects of the canine brain and nervous and endocrine systems. Elena Jazin's well-written chapter on behavioral genetics fails to live up to expectations, as the discussion on identifying genes seems to have been written for mouse geneticists. It highlights strategies for developing crosses while acknowledging that this approach is outmoded and impractical. And it fails to mention whole-genome association studies, which are likely to be the method of choice for finding genes associated with phenotypes of interest. Next, Hermann Bubna-Littitz addresses sensory physiology; of great interest is work (*5*) showing that, contrary to popular belief, the dogs' world is not black and white—they are able to discriminate at least some colors.

The book really hits its stride with Dorit Feddersen-Petersen's discussion of characteristic patterns of social behavior. Including everything from how dogs communicate during play to establishing social rights and dominance hierarchy, she highlights traits of specific breeds. For instance, Nordic breeds are better able to deal with conflict, whereas golden retrievers show social tolerance. Turning appropriately to learning, Pamela Reid covers the biology and psychology of eliciting desired behaviors as well as extinguishing unwanted behaviors through operant learning and classical conditioning.

The third section focuses on dogs and their relations with humans. One chapter is devoted to feral dogs, which, unlike wolves, lack an ordered social structure and tend to function as groups of unrelated individuals occupying defined territories. It raises some interesting questions. For example, what regulates the maximum size of a feral pack? Apparently not, as one might guess, the availability of food, but rather that the less-efficient social structure restricts the number of dogs that can effectively hunt together. In a compelling chapter on canine personality, Kenth Svartberg points out that researchers interested in individual differences in animal behavior have avoided the concept of personality because of "fear of anthropomorphism." He describes personality traits as "dispositional factors that regularly and persistently determine behaviour in many different types of situations." He notes that fearfulness is the most-studied trait in dogs, also discusses aggression and excitability, and offers an engaging section on personality formation.

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The section ends with Ádám Miklósi's excellent chapter on human-animal interactions and social cognition, which discusses in detail some classic experiments. For instance, when socialized wolf puppies are allowed to seek companionship with either a human or a dog, they generally choose the dog, whereas dogs always choose humans. Miklósi reminds us that the communication between dogs and humans goes in both directions and that dogs generally outshine wolves. For example, dogs have a harmonic version of the bark that is unknown in wolves, suggesting that dogs use barks to express a much wider range of emotions than wolves.

The last section addresses behavioral problems, especially things that can go wrong in dog-human relations. The two chapters provide descriptions related to clinical syndromes and a detailed glossary of terms that veterinary students will find useful. The coverage, however, does not extend to breed-specific diseases such as bull terrier obsessive-compulsive disorder and springer rage.

Although dog misbehavior preoccupies the typical veterinarian-client relationship, this volume aptly demonstrates that canine behavioral biology is exquisitely interesting in and of itself. No matter how they are studied, dogs continue to surprise us by fitting so comfortably into our lives. They are smart enough to interest us, affectionate enough to fill emotional voids, and clever enough to read our moods. How they do all that, and more, is really what *The Behavioural Biology of Dogs* is all about.

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10.1126/science.1144696

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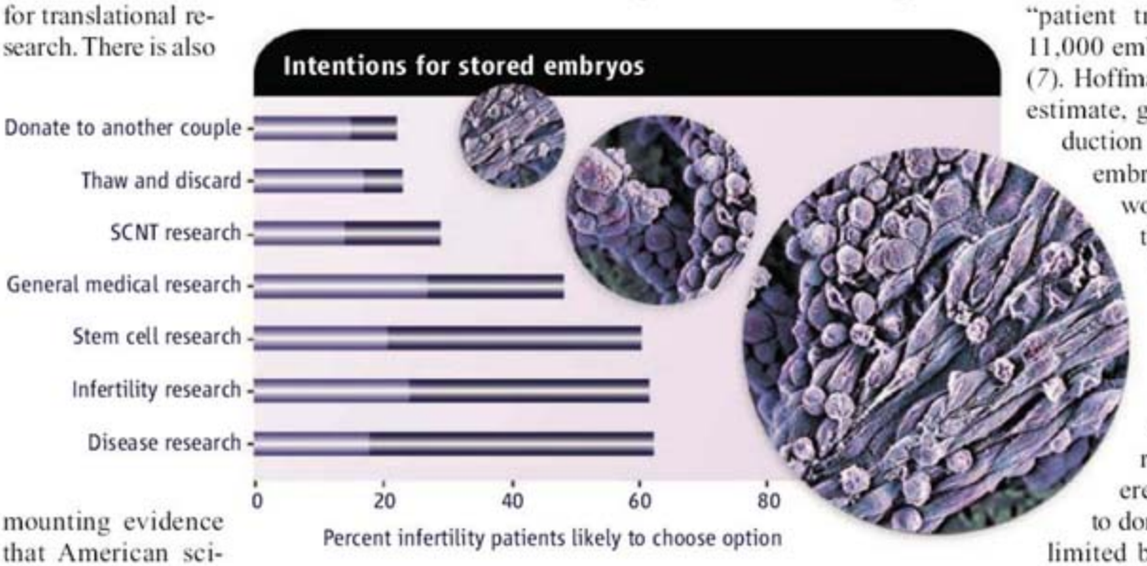
EMBRYONIC STEM CELLS

Willingness to Donate Frozen Embryos for Stem Cell Research

A national survey indicates that, for many infertility patients facing decisions about embryo disposition, research is the morally preferred option.

Anne Drapkin Lyerly[1]* and Ruth R. Faden[2]

Moral concerns about the primary source of stem cells, human embryos, have prompted one of the most contentious public debates in the history of biomedical science. Following the announcement of a restriction of U.S. federal funding to research with about 20 cell lines isolated from embryos before August 2001 (*1*) and, more recently, a presidential veto upholding this restriction (*2*), there has been a clear message from the scientific community that the eligible lines are not only inadequate in number but also unsafe for translational research. There is also mounting evidence that American scientists are losing ground to other countries with less-restrictive policies (*3*). Further, surveys of the American public indicate that there is widespread support for embryonic stem cell (ES cell) research that cuts across political, religious, and socioeconomic lines, with approval estimated at 66% of the public overall (*4*).



Disposition option for some or all of cryopreserved embryos currently stored. SCNT, somatic cell nuclear transfer. Key: Somewhat likely (lavender), very likely (gray).

Although any use of human embryos for medical research is controversial, stored embryos remaining after in vitro fertilization (IVF) treatment has been completed are widely viewed as the most morally and politically acceptable current source from which to derive ES cell lines. Many of these embryos are destroyed in any event. Moreover, although some individuals oppose the destruction of any human embryo, deriving stem cell lines from human embryos remaining after infertility treatment avoids a distinct set of particularly controversial issues associated with creation of embryos expressly for research purposes and with the use of somatic cell nuclear transfer. In fact, it is research using stem cell lines derived from such excess embryos (*5*) that would become eligible for federal funding should legislative efforts in support of human ES cell research succeed.

The potential impact of such legislation on stem cell science is at this point only speculative, however, given a less well recognized—though no less decisive—moral challenge for infertility patients whose consent must precede the ethical conduct of research on any embryo remaining after (IVF). In most cases, the embryos are placed in cryostorage because more were created than could safely be returned to a woman's uterus at the time of fertilization or in order to increase the chances of pregnancy from a single cycle of IVF. With reproductive projects completed, many patients face what is often a morally difficult task of deciding what to do with their remaining cryopreserved embryos—how to weight the options of donating them to another infertile couple, discarding them, or donating them for stem cell or other research (*6*). The results of these deliberations are largely unknown and are likely complicated by shifts in preferences over time. The only national estimate of the number of embryos in the United States came from a 2003 survey of infertility clinics by Hoffman *et al.*, indicating that, although about 400,000 embryos are currently stored, most (87%) are being held for "patient treatment" and only 2.8%, or 11,000 embryos are available for research (*7*). Hoffman and co-workers' conservative estimate, given expected loss rates in production of stem cells from cryopreserved embryos, was that only 275 cell lines would result in the unlikely situation that every donated embryo was used for stem cell research.

A major limitation of that national survey was that clinics (rather than patients themselves) were asked about embryo disposition. The only studies reporting directly on patient preferences indicate greater willingness to donate embryos for research, but are limited by low response rate (*8*), small size, and sampling from a single clinical site (*9*) and were usually conducted in populations outside the United States, including Europe and Australia. For example, cross-sectional surveys of infertility patients with embryos currently stored indicate broader acceptance than current U.S. estimates of the option of donation for research. In Australia, 30 to 44% were willing to donate embryos for research (*10*, *11*). In the United Kingdom, a prospective survey found that the option of donating supernumerary embryos was chosen by 54% of infertility patients at the time of IVF (*12*).

We conducted a survey of 2210 infertility patients receiving treatment at one of nine major, geographically diverse infertility centers and asked these patients about their intentions for the embryos they currently stored (*13*). Participating centers were located in California, Colorado, the

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District of Columbia, Maryland, Missouri, New Jersey, North Carolina, Oregon, Pennsylvania, and Virginia. The respondents were asked to answer a set of questions with one of the following: very likely, somewhat likely, somewhat unlikely, very unlikely, or unsure/don't know.

A total of 1244 patients returned the survey, for a 60% response rate overall [63% for women, 51% for nongestating partners (male or female)]; surveys were sent to only one member of a couple. We made clear at the outset that the embryo is destroyed if used for research.

Of the 1020 respondents who reported that they have embryos currently stored, 495 (49%) indicated that they were somewhat or very likely to donate their embryos for research purposes. These 495 individuals controlled the disposition of from 2000 to 3050 embryos. Even if only half of these embryos ultimately were donated, this finding suggests that around 1000 of the 3900 to 5900 embryos currently stored by the infertility patients in our sample would be available for research purposes.

Respondents to the survey expressed even greater willingness to donate embryos to research when certain characteristics of the research were specified. In particular, the percentage reporting that they would be somewhat or very likely to donate increased from 49% for medical research (in general) to 60% for research in which stem cells are derived. [Similar increases were observed for research to understand or develop treatment for human disease or injury (62%) and for research to improve infertility treatment (61%)]. Perhaps most surprising, 28% indicated that they would be somewhat or very likely to donate embryos to improve cloning techniques for medical science.

Our data suggest that it is reasonable to assume that 50% of infertility patients with cryopreserved embryos would be willing to donate their embryos for stem cell research. If only half of these embryos were to be donated, then, based on the Hoffman *et al.* (*7*) finding that 400,000 embryos are currently cryopreserved in the United States, as many as 25% of them, or 100,000 embryos, could be available for stem cell research. If we continue with the Hoffman *et al.* assumptions about the success of deriving lines from cryopreserved embryos, we can calculate that if 65% of the embryos survive the freeze-thaw process, then 65,000 embryos would be available, 25% of which (16,250) could be expected to develop to the blastocyst stage. Of these, a conservative 15% could be expected to become a viable stem cell line, resulting in roughly 2000 to 3000 viable stem cell lines, about 100 times the number of lines currently available for federal funding.

This is likely an upper estimate, because it is likely that many patients with currently banked embryos were never asked for permission to donate their embryos for stem cell research and because some of these patients may not be readily locatable for consent solicitation. In our study, 5% of individuals to whom we sent a questionnaire had moved or died, which may serve as an estimate of the percentage of infertility patients nationally who have been lost to follow-up and will not be available to provide consent for any disposition option. In addition, it may not be possible for those seeking consent for embryo research to locate reproductive gamete donors, some of whom may be unwilling to consent to research. In other cases, members of a couple may disagree about whether to donate embryos for research, although most of the respondents we surveyed believed that their partner would agree with their intentions.

When infertility patients choose research, more appears to be at issue than the belief that scientific progress justifies the instrumental use of early human life. In contrast to broad acceptance of the option of donating embryos for research, many fewer respondents in our survey expressed a willingness to donate their remaining embryos to another infertile couple. In fact, only 22% of individuals with embryos currently stored indicated that they were somewhat or very likely to donate them to another couple intending pregnancy—comparable in number to those who indicated they were likely to thaw and discard them. Thus, research emerged as the preferred option for supernumerary embryos—the reflective preference of those facing the very personal moral dilemma of what to do with embryos not used for infertility patients' reproductive goals.

The prevailing view has been that beliefs about the moral status of human embryos should be fully predictive of moral views about the acceptability of research requiring their destruction—that a respectful stance would entail giving existing embryos the chance to develop into children (*14*). But our data suggest that for most of the individuals who create embryos in hopes of having a baby, the preference is not that their remaining embryos have a chance at life, but rather that they be used in a way (research, and if not, simply destruction) that ensures that they do not.

There is a possibility, of course, that the reluctance of infertility patients to donate embryos to another couple is a prudential rather than a moral preference—that the idea of someone else gestating their embryo and raising their genetic child is experienced by these patients as intolerably worrisome, rather than as morally wrong. But qualitative work with infertility patients suggests a different moral view—that there are deep responsibilities to one's own embryos—responsibilities that preclude allowing them to develop into children without the knowledge, participation, or love of those who created them (*6*, *15*, *16*). Our data suggest that the way that infertility patients resolve the very personal moral challenge of supernumerary embryo disposition is consonant with the conclusions of the American public, the majority of whom support human embryonic stem cell research.

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MATERIALS SCIENCE

Shape Matters

When nonspherical granular systems form swarms, density is no longer well defined.

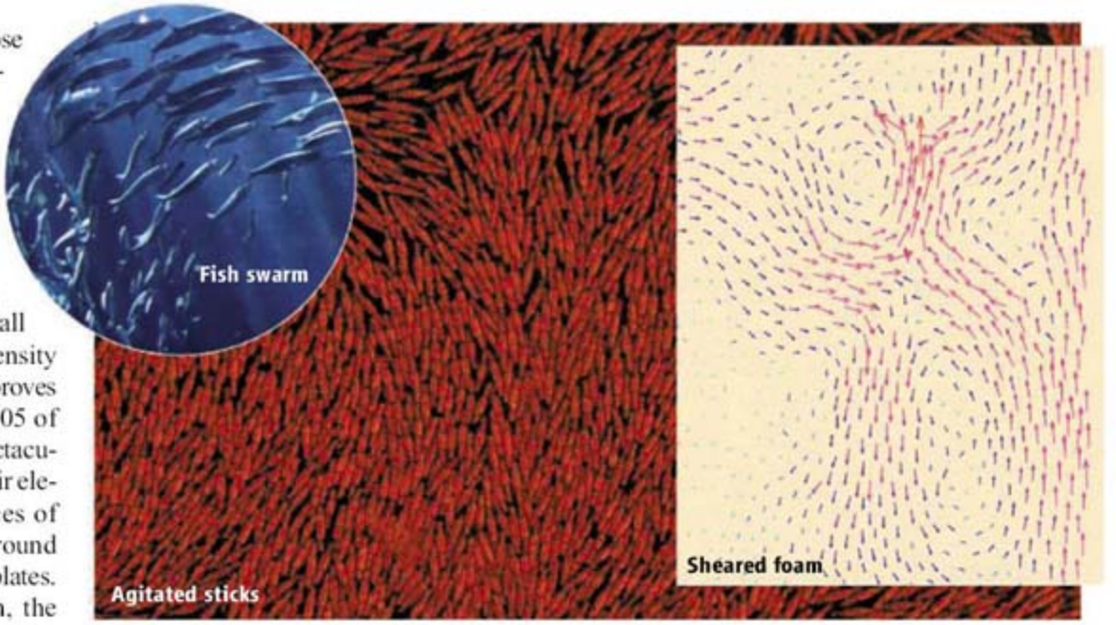
Martin van Hecke

Imagine the following: Take a pen, close your eyes, and uniformly and randomly fill a sheet of paper with dots. Now open your eyes, draw a little box on the sheet, and determine the local density of dots by counting their number in the box. You have just taken a sample—a statistical measurement used in opinion polls and blood samples alike.

Common sense suggests that the overall dot density can be estimated by their density in the sample and that this estimate improves with the size of the sample. On page 105 of this issue, Narayan *et al.* (*1*) report a spectacular breakdown of this assumption. In their elegant experiment, sticks made of pieces of copper wire are vibrated and rattle around between two closely spaced horizontal plates. Counting, in snapshots of the system, the number of sticks N in boxes of increasing size, Narayan *et al.* find that their sample-to-sample fluctuations grow proportionally to N. In other words, the local estimate for the overall density does not improve with sample size, and density is ill defined. The authors refer to this observation as giant number fluctuations.

Mathematics rules out such giant fluctuations for a broad class of systems. The central limit theorem states that when fluctuations are independent, sample-to-sample fluctuations grow only as the square root of N. This theorem applies to simple equilibrium systems (such as a gas at constant temperature) but does not automatically apply to nonequilibrium systems (such as living systems).

Narayan *et al.* use the nonequilibrium system of choice for physicists: granular materials, which are collections of macroscopic particles such as sand grains, nuts, or—in the present system—sticks. The sticklike shape is crucial for the giant number fluctuations to occur. When their overall density is large enough, nearby sticks align, something which can also be seen in equilibrium systems of elongated particles such as liquid crystals. In the stick system, alignment causes additional nonequilibrium behavior: Aligned particles propagate and form large-scale "swarms," visually reminiscent of those seen in schools of fish (see the figure). Narayan *et al.* attribute the giant fluctuations in their experiments to the complex coupling between alignment, density, and flow.



Swarms and swirls. In the experiments of Narayan *et al.*, agitated sticks form swarming states that exhibit giant number fluctuations. Similar patterns are observed in fish swarms (**top left**). Swirls are also observed in systems that are close to jamming, for example, in the motion of bubbles in a sheared foam (**right**).

Swarming and giant number fluctuations are a hallmark of the alignment displayed by driven collections of nonspherical particles. Theoretical models have been developed to describe swarming and alignment observed in schools of fish, flocks of birds, herds of sheep, or bacterial colonies—often borrowing from equilibrium models for magnetization, which consider the alignment of arrowlike objects. A very simple nonequilibrium model that exhibits cooperative motion arises when these arrows are allowed to propagate (*2*). In similar models, a collective response to predators and decision-making can arise (*3*). Toner and Tu first pointed out the giant fluctuations in such models (*4*).

In these systems, the particles have a preferred direction of propagation—just like real fish and birds. In 2003, Ramaswamy *et al.* (*5*) wondered what would happen for "active nematics," liquid crystals in which the particles have an orientation but have identical heads and tails (like the sticks in the present experiment). Their theory predicted that nematic systems also should exhibit giant number fluctuations, and these were recently observed in computer simulations (*6*).

However, when Narayan *et al.* tried to find such fluctuations in experiments, they encountered a surprising hurdle: cylindrical rods, arguably the simplest nematic particles, do not form nematic states and do not exhibit giant fluctuations (*7*). The authors achieved their present breakthrough only after etching the rods to obtain sticks with thinner ends (see the figure); for unknown reasons, these sticks exhibit nematic order. To complicate matters further, Aranson *et al.* recently performed similar experiments and observed that weak coupling between the nematic order and spurious in-plane vibrations of the support plate may strongly influence the swirling motion (*8*). Clearly, swarming is a subtle problem, and the precise nature of the swarming state and the transition to swarming is not yet fully understood.

The experiments of Narayan *et al.* are part of a bigger story, where nonequilibrium systems of nonspherical particles exhibit surprising behavior: We do not yet understand the consequence of shape. An earlier striking example of this is the finding that, contrary to expectation, M&M candies can be packed more effectively than spheres (*9*).

Imagine further increasing the density of the sticks in the experiment of Narayan *et al.* At some point, the particles will hinder each other so much that their dynamics will slow down dramatically, and collectively the sticks

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will behave as a rigid system—the system will be jammed (*10*). Studies of jamming in collections of spherical particles have uncovered many aspects of the rich structure of these disordered systems (*11*–*14*). Despite the absence of nematic order, these systems show swirling near the jamming transition (see the figure). Probing the spatial ordering and mechanical properties of nematic particles, such as ellipses, promises to provide fresh insights into swarming and jamming.

Does the transition to swarming precede the jamming transition or coincide with it? Do giant fluctuations persist near jamming? Are swarming and swirling two manifestations of the same underlying mechanism? To answer these questions, physicists will increasingly subject rice grains, rods, needles, disks, and other nonspherical objects to shaking and shearing in the coming years. What is clear already is that shape matters.

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NEUROSCIENCE

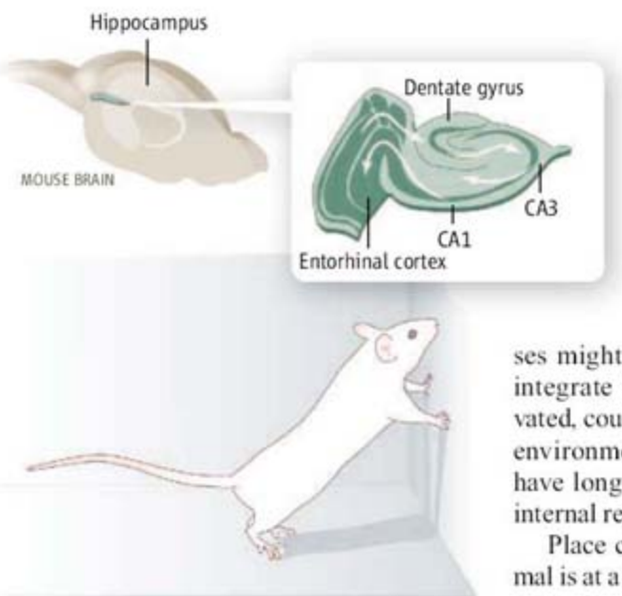
Remembering the Subtle Differences

Mice use a specific neurotransmitter receptor in the dentate gyrus of the hippocampus to detect small changes in their surroundings and differentiate between similar experiences.

David M. Bannerman and Rolf Sprengel

A good taxi driver in a cosmopolitan city has to find an arbitrary destination from any starting point, efficiently and with high precision, relying on recollection of the city's layout and taking into account changes in traffic or road conditions. The hippocampus is central to such tasks that rely on memory and spatial navigation, and the way in which it does this is a key issue in neuroscience. Over more than a decade, the Tonegawa laboratory has analyzed the function of neuronal plasticity in the rodent hippocampus by selectively altering the expression of genes associated with the function of synapses, the junctions that facilitate communication between neurons (*1*, *2*). On page 94 of this issue, McHugh, Tonegawa, and colleagues (*3*) identify an important role for synaptic plasticity in the dentate gyrus of the hippocampus for learning. The findings explain how we detect small changes in our environment, perhaps allowing us to update and guide our choices.

The principal excitatory neurons of the mammalian hippocampus are organized into three different cell layers that are linearly connected. The entorhinal cortex, which provides the major input of sensorial information to the hippocampus, sends activating signals to the granule cells of the dentate gyrus (see the figure). The dentate gyrus, in turn, sends neuronal projections (axons) to CA3 hippocampal cells. CA3 neurons project to CA1 pyramidal cells, thus establishing a "trisynaptic" pathway in the hippocampus. To complete the circuit, CA1 cells send output signals back to the entorhinal cortex. In addition to this major trisynaptic pathway, there are connections between CA3 cells and additional entorhinal cortical inputs onto both CA3 and CA1 cells. Synaptic strengths at each node in the trisynaptic pathway can be modulated, and this is partly dependent on the *N*-methyl-D-aspartate (NMDA) receptor, which is activated at synapses by the neurotransmitter glutamate. Altering the strength of individual synapses might enable hippocampal neurons to integrate into ensembles that, when activated, could represent salient features of the environment. Hippocampal "place" cells have long been taken as evidence for such internal representations (*4*).



Knowing what, when, and where. In the mouse brain, the dentate gyrus region of the hippocampus can detect small changes in the animal's spatial environment and differentiate between recent experiences that occur in the same place. The white arrows trace a path of signaling between different regions of the hippocampus. Sensory information can enter the hippocampus from the entorhinal cortex and is sent back to the entorhinal cortex after processing.

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Place cells are active only when the animal is at a particular position in space. These neurons could therefore identify the animal's current spatial location and, in concert with other neuronal ensembles, track the animal's movement. But beyond spatial information, hippocampal neuronal activity may provide a more complete representation of episodes or experiences (*5*, *6*).

Distinct features of hippocampal activity—its so-called neuronal code—are differentially sensitive to small and large changes

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in environmental or contextual features, suggesting that there are multiple mechanisms by which experiences can be differentiated (*7*, *8*). Small changes in contextual cues result in a change in the correlated neuronal activities ("rate remapping") in the dentate gyrus and CA3, whereas larger changes in contextual information result in the recruitment of different neurons, especially in CA3 ("global remapping") (*8*). McHugh *et al*. observed that rate remapping, but not global remapping, was disrupted in mice genetically engineered to lack NMDA receptors in the dentate gyrus (lack of a functional receptor prevents changes in synaptic strength). This phenotype allowed the authors to assess the role of rate remapping in differentiating between similar experiences.

McHugh *et al*. used a modified fear conditioning paradigm in which one of two experimental contexts to which mice are exposed was paired with the animal receiving a footshock. Although mice lacking NMDA receptors in the dentate gyrus acquired and retained contextual fear conditioning—the animals learned to associate fear with a particular environment—they were slower compared to wild-type littermates in learning to discriminate between two similar contexts. This result is consistent with the idea that synaptic plasticity at entorhinal cortex–dentate gyrus synapses is important when spatial context is used to identify the appropriate memory (receiving a footshock or not), and subsequently for making the appropriate behavioral response (whether or not to become immobile) (*9*). This notion has often been considered in terms of an ability to recall "what" happened "where," and could make a key contribution to episodic memory in humans. The NMDA receptor–mutant mice could, however, acquire spatial memory when learning to navigate a watermaze. The clear implication, therefore, is that NMDA receptor–dependent spatial pattern separation in the dentate gyrus is not required to solve the watermaze. So when is pattern separation a key feature of hippocampal function?

McHugh *et al*. show that subtle changes in the spatial context are sufficient to require pattern separation, but are such context shifts necessary? In a similar study, mice lacking NMDA receptors in the dentate gyrus learned to discriminate between which arms of a radial maze contain food rewards and which arms are not rewarded: They acquired spatial "reference" memory, as did the mice in the watermaze experiment (*10*). However, the mutant mice could not keep track of which arms they had already visited, making more spatial "working" memory errors than wild-type mice with intact dentate gyrus physiology. The requirement for spatial pattern separation is the same for both the reference and working memory components of this task. Therefore, impaired spatial pattern separation cannot explain this spatial working memory deficit.

To solve a spatial working memory task, an animal must also process the temporal context associated with an event ("what happened, and when") and record or represent which locations have been visited recently. A role for the hippocampus in encoding temporal sequences in rodents has been demonstrated (*11*). Now, the new studies with genetically modified mice show that NMDA receptor–dependent plasticity at synapses in the dentate gyrus, and the possible subsequent rate remapping of correlated neural activities, contribute to temporal information processing, providing a mechanism by which recent experiences could be represented and thus differentiated.

The development of mice with inactivated or activated genes in specific regions of the hippocampus constitutes a great step forward in our understanding of how the hippocampus works. The challenge now is to understand the roles of individual NMDA receptor subunits, different forms of synaptic plasticity (long-term potentiation, long-term depression, and depotentiation), and the various hippocampal subregions (dorsal-posterior and ventral-anterior) in regulating behavior.

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CANCER

Sex, Cytokines, and Cancer

Toby Lawrence, Thorsten Hagemann, Frances Balkwill

A cell signaling protein associated with the innate immune response is linked to a pathway that supports cancer progression.

Cancers are not just malignant cells. More than half of the cancer mass can be made of supporting cells such as fibroblasts, tissue macrophages, and endothelial cells; cancers cannot progress into life-threatening metastatic lesions without them. The process by which normal cells are recruited, expanded, and maintained in cancers is closely related to inflammation and to the remodeling that occurs in tissues as the damage of acute inflammation is repaired (*1*). Two papers in this issue, by Naugler *et al*. on page 121 (*2*) and Rakoff-Nahoum and Medzhitov on page 124 (*3*), advance our understanding of the mechanisms of cancer-related inflammation. They describe an important role for an intracellular signaling protein called MyD88 in the development of experimental liver and colon cancers in mice. MyD88 function has been well characterized in the innate immune response (*4*), relaying signals elicited by pathogen-associated molecules and by the inflammatory cytokine interleukin-1 (IL-1). Its identification in promoting cancer progression reveals a molecular pathway that could be targeted for drug development.

Early experiments demonstrated the need for inflammatory cytokines such as tumor necrosis factor–α (*5*), and inflammatory cells such as macrophages (*6*), in the development and spread of some experimental tumors. More recently, activation of the transcription factor nuclear factor κB (NF-κB), which is critical in cellular responses to TLR ligands and IL-1, was implicated in the innate immune response promoting murine hepatocellular and colon carcinoma (*7*, *8*). The conclusion from Naugler *et al*. and Nahoum and Medzhitov is that MyD88 may function upstream of NF-κB in cells

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involved in inflammation-associated cancer (see the figure).

A key finding of Naugler *et al.* is that chemically induced liver damage in mice leads to the MyD88-dependent induction of IL-6 production. In this liver cancer model, IL-6, made by liver macrophages (Kupffer cells), promotes tumor progression. By specifically eliminating NF-κB activation in macrophages, this group previously established that during the development of liver cancers, IL-6 secreted by Kupffer cells requires NF-κB activity. MyD88 is required for both TLR and IL-1 to activate NF-κB in the innate immune response (*4*). The data of Naugler *et al.* suggest that TLR ligands (or IL-1β released by dead hepatocytes) could drive NF-κB activation in Kupffer cells through MyD88. However, the selective ablation of MyD88 in Kupffer cells is required to firmly establish this link. Ablation of specific TLRs or the IL-1 receptor would also reveal whether inhibition of these receptors could protect against liver tumor development.

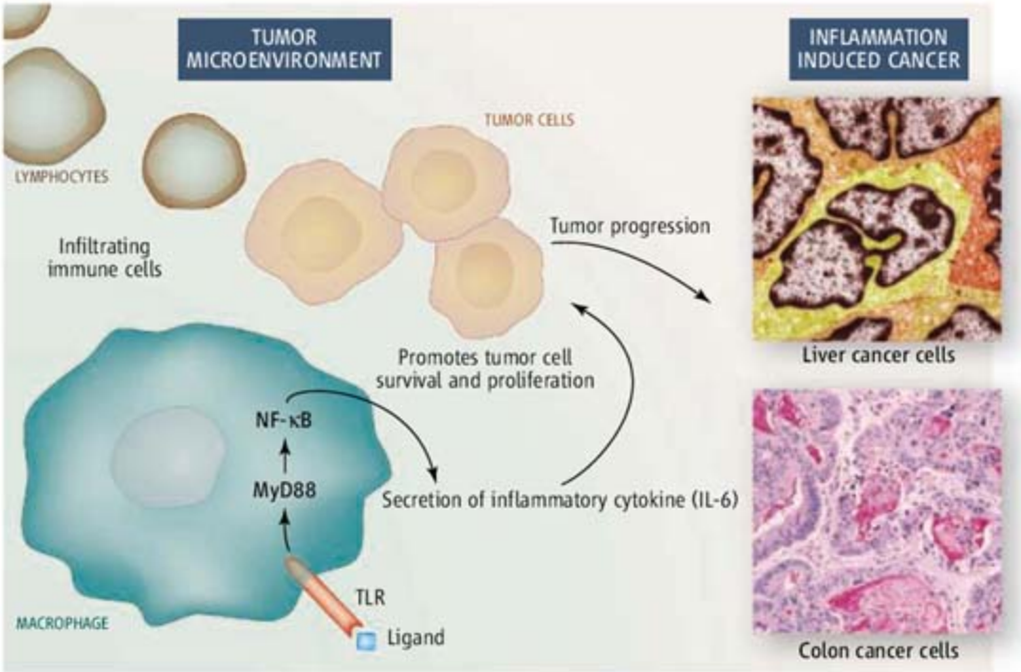
Although the study by Naugler *et al.* implies that IL-1 or ligands for TLRs may trigger MyD88 activity and an innate immune response in liver cancers, it is likely that activation of MyD88 in spontaneous colon carcinogenesis, as described by Rakoff-Nahoum and Medzhitov, is driven by commensal bacteria that encounter intestinal macrophages. These authors crossed MyD88-deficient (*Myd88*$^{-/-}$) mice with mice that spontaneously develop intestinal tumors due to a mutation in the adenomatous polyposis coli (*APC*) gene (ApcMin/+). Mortality of the resulting mice was reduced by more than 60%. Although the absence of MyD88 did not affect initiation of malignancy, microscopic tumors failed to progress, and production of many inflammatory and tissue-remodeling factors (including IL-6) decreased. This study did not determine the role of MyD88 or NF-κB signaling in the intestinal macrophages of the ApcMin/+ mice. However, Greten *et al.* (*7*) have shown that ablation of NF-κB activation in macrophage cells protect mice from chemically induced colon carcinomas.

It's important to note that NF-κB also has cytoprotective and anti-apoptotic functions in malignant cells (*7*, *9*). However, neither of these phenotypes appears to be MyD88 dependent, suggesting that MyD88 is only responsible for inflammation-associated tumor promotion by NF-κB.

Another key finding of Naugler *et al.* relates to the gender differences in liver cancer susceptibility. In their model, the actions of IL-6 were only seen in male mice. Male mice—and men—are three to five times as likely to develop liver cancer as females because estrogens are key inhibitors of IL-6 production. At all ages, women are less likely than men to develop colon cancer (*10*). Estrogen hormone replacement therapy also reduces the incidence of colorectal cancer in postmenopausal women, and in several murine colon cancer models, females have fewer colon tumors than males. These estrogen-related gender differences may well extend to other cancers.

However, MyD88 seems to have a wider role in malignancy than regulation of IL-6 production. Although Rakoff-Nahoum and Medzhitov do not specify the age and sex characteristics of the mice used in their experiments, they present no evidence that only males were protected from colon cancer when MyD88 was deleted. In ApcMin/+ mice (presumably both male and female), MyD88 induced the expression of factors (the enzymes COX-2 and MMP-7, and IL-1) that not only enhance tumor progression, but also are involved in tissue repair. It seems that MyD88 regulates a tissue-repair pathway that both promotes, and is triggered by, growing malignancy.

Do these experiments in mice have any relevance to cancer prevention and therapy? As suggested by Naugler *et al.*, estrogen mimetics that inhibit excessive IL-6 production might prevent chronic liver disease in men. Certainly, TLR ligands are possible targets for cancer therapeutics, but their inhibition could severely compromise immune responses. The findings of Naugler *et al.* and Rakoff-Nahoum and Medzhitov strengthen the rationale for inhibiting inflammatory cytokines in cancer. IL-6 is implicated in the progression of several other human cancers including multiple myeloma, prostate cancer, and ovarian cancer. Antibodies that inhibit IL-6 action are safe and active in rheumatoid arthritis patients (*11*). They are also highly effective in the IL-6–driven myeloproliferative disorder Castleman's disease and are currently in Phase I/II clinical trials in other advanced cancers (*12*). But will these agents work best in males and postmenopausal women?



Inflammation spurs cancer progression. Immune cells infiltrate the microenvironment of a tumor. Naugler *et al.* and Rakoff-Nahoum and Medzhitov suggest that the development of liver and intestinal cancers in mice may depend on a signaling pathway in infiltrating immune cells that involves the protein MyD88, the transcription factor NF-κB, and the pro-inflammatory cytokine IL-6. Shown are images of human cancers.

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COMPUTER SCIENCE

Virtually Trustworthy

How online personas are designed may increase the impression of trustworthiness, but that does not make the information conveyed more reliable.

Judith Donath

Our impression of others in face-to-face interactions derives from multimodal observations: We hear voices, see faces and gestures, and listen to each other's words. Our online interactions are less sensory, dominated instead by text: We send e-mail, read discussion boards, and participate in text chats. Yet that may be changing. Online 3D spaces such as *There* and *Second Life* feature detailed virtual environments in which users represent themselves with graphical avatars, often highly customizable.

Avatars may closely resemble a person, or they may be fantastical creations such as aliens or talking lizards. Their online use dates back to the mid-1980s, when they were used in games such as *Ultima* and in the online social space *Habitat*. As technology has advanced, they have gone from being static 2D images to complex 3D animations, complete with realistic gait, fashionable clothing, and dynamic facial expressions.

The challenge in creating more sophisticated avatars lies partly in the domain of computer graphics, such as better rendering of hair and fabric or more lifelike gait kinematics. Yet there is also a substantial social element: getting the avatar to interact with others gracefully and realistically. For example, if an avatar is rendered with detailed eyes, then appropriate gaze direction is essential. All such behaviors, which are taken for granted in face-to-face interaction, must be explicitly coded into the avatar.

We carry out social interactions with a large number of communicative behaviors that indicate our intention, state of mind, communicative competency, and so on. For instance, you may see an acquaintance across the room at a cocktail party and decide to go speak to him. You carry out this goal not only by walking across the room but also by making eye contact, smiling, raising your brows, adjusting your clothes—a complex set of communicative behaviors that indicate your intention to start a conversation, allow you to gauge his willingness to do so, and show your level of determination. Cassell and Vilhjálmsson (*1*) argued that avatars without these social behaviors seem stilted and awkward. They can be moved to stand next to each other to talk, but stare blankly into space, inert and unengaged.

Just giving the user finer control over the avatar is not a satisfactory option, however, for if users must specify their avatar's every eye movement and gesture, they would be too distracted to engage in the conversation itself. Cassell and Vilhjálmsson's solution was to program such behaviors into the avatars, to be set off when the user indicated some desired action. For instance, if the user indicated (with a simple typed command) that she wanted to end a conversation, the avatar would break away by averting its gaze, and upon leaving would look at its recent partner, nod its head, and wave. Users of this system found it more natural and engaging than one with static avatars and felt that their conversational partners were more expressive.

Researchers have found that users infer a number of character traits from avatar behavior and appearance. They judge avatars that are humanlike and clearly gendered (as opposed to androgynous) to be the most attractive and the most credible (*2*). In an audio-only conversation, simply adding an avatar whose head and eye movements match the conversation flow increases users' perception of their partners' trustworthiness and friendliness.

Today's online graphical interactions are still rather awkward. Behavioral sophistication lags behind rendering skill, so we have avatars whose appearance raises high expectations of humanlike behaviors but whose gaze and gestures are relatively primitive. However, it is quite conceivable that in a few years avatars whose behavior is nearly imperceptible from humans' will be available.

Held in trust. Self presentation in real and virtual life.

CREDIT: DIETMAR OFFENHUBER

Yet this raises important questions about the reliability of the impressions we form in avatar-mediated interactions. In our face-to-face interactions, many of the cues we read to assess traits such as trustworthiness have real links to the trait. Gaze direction, for example, links directly to what one is seeing. When long averted, it is thus a sign of inattention. During times of high cognitive load, such as when inventing a story, people may make less eye contact, possibly because gazing at another face is itself a cognitively intensive process. Perhaps because of this, we have the popular (though unsubstantiated) belief that someone who makes steady and direct eye contact is being honest (*3*).

Online, however, behaviors generated by a software program can create the same impression of trustworthiness or friendliness, but without a grounding connection to an underlying cognitive process or other causative element. As behavioral software becomes more sophisticated, are we creating avatars that will be increasingly attractive and seemingly friendly but are in fact the ideal mask behind which a dishonest or manipulative person can operate? Once an interface includes humanlike avatars, the issue of user interpretation of character traits from ungrounded avatar behaviors is inevitable, for even nonaction is an interpretable behavior; it conveys an impression of social ineptness and distance.

How we assess these issues depends on the context in which they are used and how we view them in relation to real-world practice. In the real world, we use many strategies to enhance the impression we make on others. We employ resume consultants and speech coaches, wear makeup, and undergo plastic surgery. In the virtual realm, idealized bodies and perfect skin are the norm (*2*), but there are also a whole new range of possible enhancements. Bailenson and colleagues have conducted several experiments on digital mimicry, such as morphing a person's own face into the avatar of their conversational partner or having that avatar closely mimic their ges-

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tures (*4*). They found that people in a group paid closer attention to messages delivered by a "team face" avatar, made by combining the features of the people in the group. This could be a useful technique for enhancing the cohesiveness of geographically dispersed groups. Yet they also found that politicians' arguments were more persuasive when their faces were made to subtly resemble the listener's, raising the specter of a world in which you are bombarded with oddly compelling ad campaigns presented by people just like you.

Reliability involves tradeoffs. Less reliable communication is often cheaper or easier; when deception becomes too prevalent, more costly signals or social sanctions may be needed (*5*). Suspicious citizens of the future may demand to interact with candidates only through trusted "manipulation-free" sites. Today, most 3D graphical sites are fantasy games, where role-playing and artifice are not only accepted but also required. As social sites such as *Second Life* gain popularity, other uses are emerging, including academic lectures, retail stores, and business meetings. These will require a range of avatar designs, not only in terms of technical sophistication but also across a continuum from the most attractive and impressively persuasive to the most rigorously and reliably grounded.

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CHEMISTRY

Shining Light on the Rapidly Evolving Structure of Water

Time-resolved infrared spectroscopy is shedding light on the dynamics of hydrogen bonding in liquid water.

Andrei Tokmakoff

The molecular origins of the physical properties of water continue to puzzle scientists. Each tool provides only a limited perspective, revealing certain aspects of the hydrogen-bonding structure or of the ultrafast time scales over which the structure changes. Now, a new generation of time-resolved vibrational spectroscopies is providing detailed insights into how the structure of water evolves. The results raise questions about the nature of hydrogen bonding.

The structure of liquid water is generally conceived as a disordered network of molecules connected by hydrogen bonds (*1*). This structure fluctuates and reorganizes on time scales between 10 fs (10^{-14} s) and 10 ps (10^{-11} s). This hydrogen-bond dynamics is at the heart of the unique physical, chemical, and biological properties of water. Insights into its structural properties have come from x-ray and neutron-scattering experiments, which lack dynamical information; insights into its dynamics have been gained from ultrafast time-resolved experiments, which have lacked structural detail. The most detailed understanding of liquid water derives from molecular dynamics simulations, which commonly treat the liquid as rigid molecules with charges. Such simulations provide an atom-by-atom perspective on how hydrogen bonding changes with time, but their dynamics have never been properly tested against experiment.

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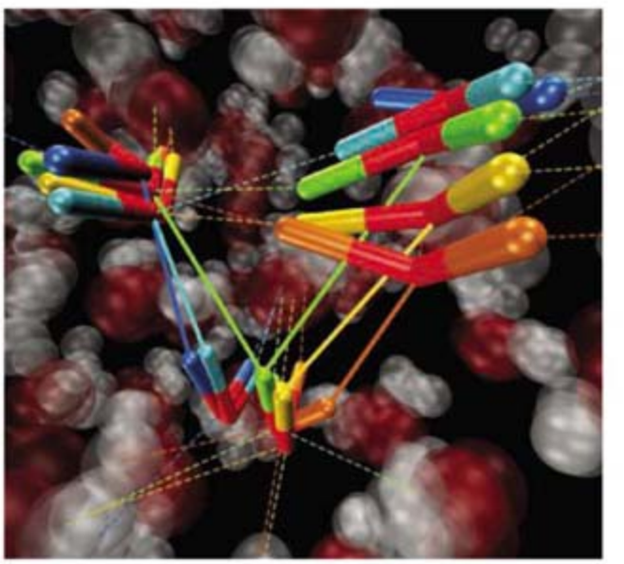
Femtosecond infrared spectroscopy bridges the gap between these methods by providing a structure-sensitive probe of how the hydrogen-bond network in liquid water evolves. In these studies, hydrogen bonding is probed by monitoring the frequency of the O-H bond–stretching vibration, which decreases with increased strength of the hydrogen bond in which it participates. The newest method of two-dimensional infrared spectroscopy (2D IR) uses ultrafast infrared light pulses to track how the frequencies of different O-H bonds evolve with time.

However, spectroscopy cannot tell you everything. Simulations are important for providing the structural interpretation of the experiments, drawing on a theoretical description of how the O-H frequency is determined by hydrogen-bonding structure (*2*–*4*). This interpretation tool has initiated a feedback process in which the simulation describes how structural changes appear in the experiment, and the experiment provides the benchmark for the computer model.

Asbury *et al.* were the first to perform 2D IR experiments on water (*5*). By studying the isolated O-D vibration of dilute HDO in H_2O (D is the ^2H isotope), they mapped the time scales over which a water molecule samples different hydrogen-bonding configurations. They observed dynamics on time scales of 48 fs, 400 fs, and 1.4 ps, and attributed the short times to fluctuations in the hydrogen bonds and the long times to hydrogen-bond breaking and forming. These results were qualitatively similar to simulations, but the time scale for hydrogen-bond breaking was nearly two times slower than for the widely used SPC/E and TIP4P water models. Fecko *et al.* made similar observations for HDO in D_2O using the related technique of echo peak shift spectroscopy. In addition to 50-fs fluctuations,



Switching a hydrogen bond. In this 288-fs sequence taken from a classical simulation, structural fluctuations induce the switching of a hydrogen bond between the donor molecule (bottom) and an acceptor molecule (upper left) to a new acceptor (upper right). The hydrogen-bond connectivity is color coded, showing the rotation from the initial acceptor (blue) through the bifurcated state (green) to the approaching new acceptor (orange).

they observed hydrogen bonds vibrating with a period of 200 fs, and network reorganization on a 1.4-ps time scale (*4*).

More recent 2D IR experiments on pure H_2O have revealed a different type of dynamics: the flow of vibrational excitation through many water molecules. The O-H vibrations in the liquid are collective vibrations spread over many molecules. These collective vibrations result from efficient intermolecular energy redistribution, which Cowan *et al.* have shown occurs within an extraordinarily fast 50 fs (*6*). Furthermore, the vibrational excitation energy in the O-H bond cascades rapidly into bending vibrations, hindered molecular rotations, and heating of the surroundings within less than 1 ps (*7*, *8*). Thus, the dynamics of water provide an unusually effective route for dissipating vibrational excitation, the energy relaxation process that guides the conversion of reactants to products in aqueous chemical reactions.

By which mechanism does the structure of liquid water evolve with time? Simulations have described picosecond rearrangements between "inherent structures"—groups of molecules with a certain hydrogen-bond connectivity about which the structure fluctuates (*9*). Recent studies have sharpened the picture. With 2D IR, Eaves *et al.* watched adjacent molecules that did not appear hydrogen bonded, and showed that all of these species re-formed a hydrogen bond within 150 fs (*10*). This demonstrated that liquid water does not contain "dangling bonds" that persist as an intermediate in the switching of hydrogen-bonded structures. The liquid reorganizes through abrupt, concerted events that switch a hydrogen bond from one partner to another.

Simulations by Laage and Hynes provide a detailed mechanism for this process (*11*). They found that switching involves the concerted lengthening of a hydrogen bond between donor and acceptor, accompanied by rotation of the donor's O-H bond toward an approaching new hydrogen-bond acceptor (see the figure). At the transition state, the donor hydrogen bond is bifurcated between the two acceptors. This switching occurs when the initial acceptor experiences a momentary over-coordination by hydrogen bonds and the new acceptor is under-coordinated.

An unanswered question lurks behind all studies of water: What is a hydrogen bond? Researchers currently use working definitions: for instance, a geometric criterion in simulations or a distinguishing spectral feature in experiments. The use of such nontransferable, yes-or-no definitions to describe a phenomenon that varies smoothly in space and time has led to many arguments about how to reconcile different studies.

Is it possible to do better? There are limitations to interpreting water properties in terms of hydrogen bonding, as detailed ~40 years ago by Eisenberg and Kautzmann (*12*). Perhaps researchers should focus on more tangible observables such as nuclear position and electron density. Or perhaps a dynamical definition of a hydrogen bond will help. It is only meaningful to refer to a bond as stable if it persists for longer than a vibrational period, a characterization possible both by experiment and simulation. The lack of clarity in these matters is an example of what makes water an enigmatic substance.

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10.1126/science.1144515

APPLIED PHYSICS

The Power to Set You Free

Wireless power transmission may allow computers, cell phones, and other devices to be completely free of the need to plug into a wall socket.

Will Stewart

One of the best-known technology magazines is *Wired*, capturing in one word both the attraction and the bane of the Information Age. We all want to be connected, but none of us loves the cables that connect. Of course, a rapidly expanding plethora of wireless technologies—cellular phones, WiFi, ID tags, Bluetooth, and many others—provide data connectivity. But despite improvements in battery technology and Moore's Law, the increasing performance of portable devices still has us reaching for a power cord far more often than we would like. But now Kurs *et al.* report on page 83 of this issue an ingenious approach that may offer us a chance for true wireless freedom (*1*).

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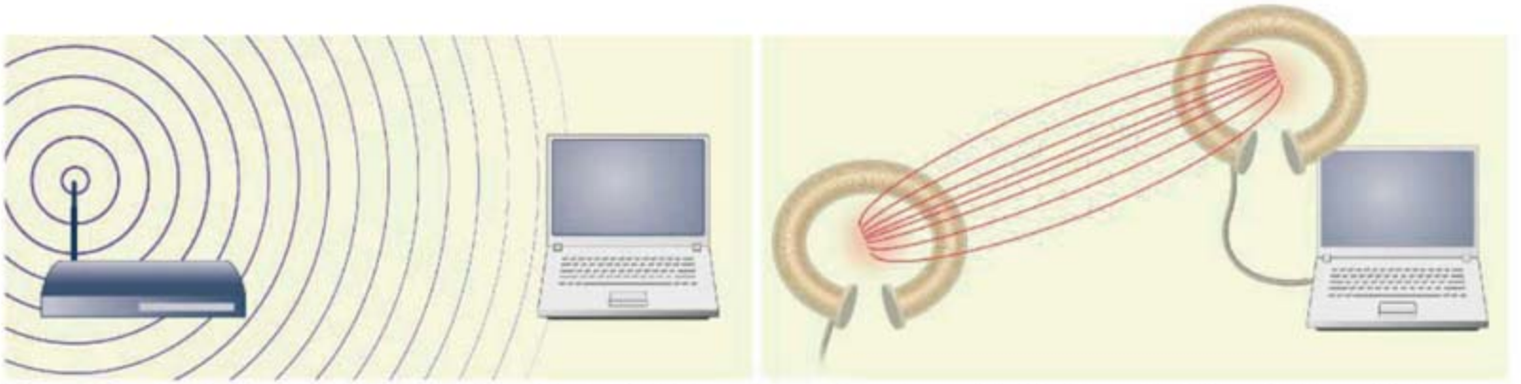
Wireless data are so pervasive (my laptop can see nine local wireless devices from my seat on a train as I write) that readers may wonder what is so difficult about providing a wireless power cord. And one can transfer power as well as information by means of electromagnetic radiation; indeed, sunlight is the ultimate source of most of the energy we use. Sunlight can be extraordinarily intense, but it is still barely enough: The sunshine falling on my laptop carries about 25 W, about half of what is needed.

By wireless communications standards, on the other hand, sunlight is very bright. A typical WiFi station radiates only ~100 mW, a fraction of 1% of the wattage needed to power my laptop. This has two consequences: (i) If wireless power were simply sprayed out for anyone to collect as if it were sunlight, even a short-range power source would have a prodigious power consumption. (ii) Such a source might represent a hazard to health (a much more reasonable worry than current concerns over WiFi).

Getting around these issues is tricky. There have been a number of moderately successful efforts to make working systems (*2*), mostly based on near-contact (i.e., centimeter-range) power transfer. These use the sort of magnetic field induction found in a transformer or an induction motor, both of which rely on a nonradiating "evanescent" field that reduces the power lost to radiation. But the power transfer falls off very steeply and the range is very short. The result is a powered pad on which a suitably enabled device can be placed to charge—wireless indeed, but not very mobile.

Kurs *et al.* work around this by making both the "sender" and the "receiver" of electrical power operate at the same moderate radio frequency. With carefully chosen



Going cordless. (**Left**) A conventional radio transmitter, such as in a WiFi link, emits waves that weaken rapidly with distance. (**Right**) Kurs *et al*. have devised a system for transmitting power where the energy flow is much more directed, which produces higher efficiency.

parameters, the two coils form a single coupled resonant structure and behave as though a "tunnel" was opened between them that can carry substantial power over ranges of several meters (see the figure).

The decay in the coupling between the source and receiver with increasing source-receiver separation is still quite steep relative to sunlight-style radiative transfer. However, this no longer translates directly into a decay of power transfer efficiency, because untransferred power remains trapped around the source and all the power could still be transferred with ideal components. This technique cannot in reality extend the range indefinitely—for example, the power trapped around the source will tend to rise unacceptably, and imperfect real components will cause losses—but it does help a lot (compare figures 2 and 4 in Kurs *et al*.).

And there is another likely benefit from the use of these resonances, as suggested by Pendry (*3*), which addresses the possible health concern. Unlike a freely propagating electromagnetic wave (such as sunlight), where the electric and magnetic components are always of similar intensity on average, these resonances are overwhelmingly magnetic in character. This could be extremely helpful in reducing the hazard to health, because most ordinary materials (including people) interact far more strongly with the electric than with the magnetic component of an electromagnetic wave, so the absorbed power can be much less for a given amount of power transferred. This helps efficiency but, far more important, it reduces the microwave oven–style heating within brain tissue that defines the known hazard limits for all radio-frequency devices such as mobile phones. This effect has not yet been proven by standard safety tests, but it looks very promising.

Although the system now works and transfers a useful 60 W of power over an impressive 2 m, there are still some important issues that need addressing. For example, of the 60 W of power transferred, ~5 W currently ends up as unintended radio radiation. This may not seem much as a proportion of the power transferred, but it is still a lot of radio power; a cellular handset, for example, is limited to much less than this. But as Kurs *et al*. point out, this can be reduced by improving the components and the design to keep the external fields more purely magnetic, possibly enabling further reduction of health hazards. And interference effects may prove manageable; we note, for example, that interference caused by unintended leakage from high-speed radio-frequency Internet transmission lines has been less of an issue than was once feared. There may still be issues with loss of power and induced radiation caused by metal and other objects that happen to be lying around within the field, although the preliminary experiments here look promising and the resonant nature of the transfer should help. And improvements, particularly in nonemissive displays (*4*), may mean that portable device power requirements may begin to fall. So we are not wire-free yet, but revolution is in the air.

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10.1126/science.1145008

RETROSPECTIVE

Robert W. Cahn (1924–2007) and David Turnbull (1915–2007)

Roger Doherty

This is in remembrance of two major figures in the development of modern materials science.

Sixty years ago, the precursor discipline to materials science—metallurgy—was largely empirical, necessarily so given its engineering importance and complexity. However, by the late 1940s, the field was about to be transformed. Robert W. Cahn and David Turnbull, who both died in April of this year, played vital roles in the development of this new science.

The two men came from remarkably different backgrounds. Cahn was born into a prosperous and artistic Jewish family in Fürth, Germany, on 9 September 1924. The family fled Nazi Germany in 1933, and in 1942, Robert entered Cambridge University (*1*). Turnbull was born on 18 February 1915 on a family farm in Elmira, Illinois. His family rarely ventured more than a few miles from their farm (*2*). He graduated from high school in 1932 at the height of the Depression, but was able to attend Monmouth College, which was governed by the Presbyterian sect to which his family belonged. Here he became interested in physical chemistry and went on to a scholarship and a Ph.D. at the University of Illinois in 1939.

Cahn's major contributions to the new science of physical metallurgy started during his graduate research, where his experi-

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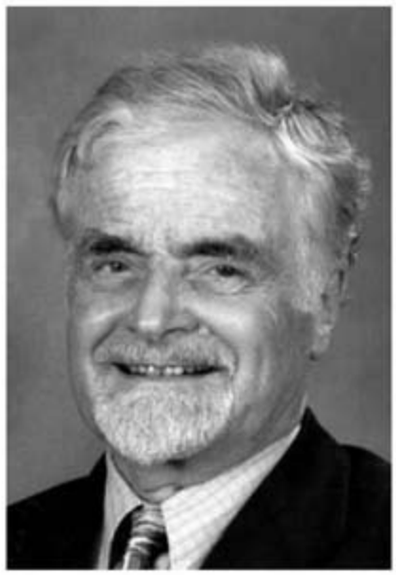
ments demonstrated the reality of dislocations (line defects whose movement under stress allows metals to deform plastically). Dislocations had been proposed to explain the discrepancy between the very high predicted resistance to plasticity of perfect metal crystals, compared to the much smaller measured values of actual pure metal crystals. In pure metal crystals, dislocations can move under the influence of very small shear stresses. Metal processing techniques had always used solute atoms, second phases, and other obstacles (which impede the movement of dislocations) to improve the metals' resistance to plastic deformation and to control brittle fracture. Until the advent of dislocation theory, however, the question of what controls the properties of metal alloys such as steel could not be addressed scientifically.

From his graduate student work, Cahn also proposed a successful model for the nucleation of new crystals formed during recrystallization, a softening process that occurs when heavily deformed metals are heated, allowing them to be reshaped into new objects. In his model, the dislocations introduced by previous deformation rearranged to create small regions of dislocation-free crystals that can grow to become new grains. This model provided the basis of almost all subsequent research into this important industrial process, which is used to control the size and orientation of grains in metallic alloys to improve their strength and ability to be shaped, for example, in automobile bodies made from pressed steel sheets.



Robert W. Cahn

David Turnbull

Turnbull made one of the major discoveries in materials science after joining the GE research laboratory in 1946. His elegant investigations into the nucleation of structural transformations (initially the solidification of liquid metals) showed that such complex processes could be quantitatively understood. The theory for this type of process had been developed a decade or so earlier by physical chemists studying crystallization from supersaturated liquid solutions. The initial formation of a crystal—for example, of salt from an aqueous salt solution—was known to require highly supersatured conditions, because the first small crystal to form, being chemically different from water solution, has a large interfacial energy. Yet, ingots of metals needed only a very small supercooling (a temperature below the melting temperature) to start to form solid metal crystals. The understanding was that the interfacial free energy between a metal crystal and its melt must therefore be small.

Turnbull carried out experiments on the freezing of a low–melting-point metal, gallium, and found that the small supercoolings usually seen resulted from particles of dirt, more politely called "heterogeneous catalysts." By breaking up the liquid gallium into small droplets, so that there were more droplets than there were pieces of dirt, he achieved the very large supercoolings characteristic of what is called "homogeneous" nucleation. His result indicated a large interfacial energy between crystalline and liquid gallium. In the early 18th century, Fahrenheit had reported similar observations for the freezing of water. Turnbull was now able to explain what was happening and show its universal application.

To show that this principle also applied to metals with simple crystal structures, such as iron or copper, Turnbull, together with R. E. Cech, developed a simple experimental means of studying the phenomenon. By heating small droplets of high–melting-point metals, such as copper, on an inert substrate and then cooling them below their melting temperatures, they demonstrated very large undercoolings.

Through these experiments and theoretical insights, the previously empirical study of metal solidification had acquired a clear scientific foundation. Turnbull and his colleagues at GE went on to develop the scientific discipline of metal-alloy processing. In a particularly important later study, he and I. S. Servi (*3*) demonstrated the validity of homogeneous nucleation theory for a solid-solid transformation: the nucleation of coherent cobalt particles from dilute solid solutions of cobalt in copper. Solid-state nucleation processes form the basis of the technologically vital process of strengthening metallic alloys by precipitation hardening.

Both of these pioneers made many other vital contributions. Cahn studied the crystallography of uranium, mechanical twinning, and ordered intermetallic alloys. Turnbull made important contributions to solid-state diffusion and the structure of liquids. Both scientists also studied metallic glasses (metallic alloys that can be cooled to form rigid noncrystalline solids). Their research was instrumental in the development of today's approach to materials science, in which the structure of materials is modified to produce new and improved properties—such as higher strengths or improved electrical and magnetic properties—needed for applications ranging from aircraft engines to information-storage devices.

Cahn was a successful academic at several universities in the United Kingdom and in France, as was Turnbull at Harvard after he left GE in 1962. Cahn also made great contributions to publishing, particularly the encyclopedic tome *Physical Metallurgy* that he co-edited with Peter Haasen. Turnbull, in his research and teaching, was always a materials scientist curious about any material with interesting structural problems, independent of its chemical bonding. Sadly, Turnbull never translated his lecture notes on structural transformations into a textbook. Those of us who have written such textbooks are free of a major competitor, but his textbook would have been a contribution that we regret not having available to us.

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10.1126/science.1145490

Stability and Diversity of Ecosystems

Anthony R. Ives[1] and Stephen R. Carpenter[2]

Understanding the relationship between diversity and stability requires a knowledge of how species interact with each other and how each is affected by the environment. The relationship is also complex, because the concept of stability is multifaceted; different types of stability describing different properties of ecosystems lead to multiple diversity-stability relationships. A growing number of empirical studies demonstrate positive diversity-stability relationships. These studies, however, have emphasized only a few types of stability, and they rarely uncover the mechanisms responsible for stability. Because anthropogenic changes often affect stability and diversity simultaneously, diversity-stability relationships cannot be understood outside the context of the environmental drivers affecting both. This shifts attention away from diversity-stability relationships toward the multiple factors, including diversity, that dictate the stability of ecosystems.

Stability has a rich history in ecology. Theoretical research has explored how numerous features of ecosystems affect stability, including diversity (number of species), the strength of interactions among species, the topology of food webs, and the sensitivities of species to different types of environmental perturbations. Empirical studies have generally focused more specifically on diversity, particularly in the past 15 years. This is because diversity is easier to measure and manipulate than other features of natural ecosystems, and because such research is relevant to the debate about the worldwide loss of biodiversity (*1*, *2*).

Historically, the relationship between diversity and stability has been contentious. Different theoretical results contradicted each other, empirical results were inconsistent, and theoreticians and empiricists often disagreed. Although the storm has begun to subside, we fear that ecologists risk becoming complacent about the diversity-stability debate. Are we asking the right questions about diversity and stability? Are we asking them in the right way? Our goal here is not so much to answer these questions as to show that they still need to be asked.

Concepts of Stability

A fundamental problem in this context is that stability can have many different definitions (*3*–*5*), and each definition gives a different diversity-stability relationship. Different theoretical concepts of stability apply, depending on the type of inherent dynamics exhibited by a system and the type of perturbation the system experiences. Here, we give an overview of some types of stability (*6*). We focus on concepts of stability that involve some integrated measure of the entire ecosystem, such as the summed density of all species, rather than species-level measures; these are not generally independent, but neither are they completely inseparable (*7*).

Systems may have alternative stable states (Fig. 1A), in which the final densities of species, or even the persistence of species, depend on their initial densities (*8*–*10*). For example, Scheffer *et al.* (*11*) showed that shallow Dutch lakes can occur in either a clear-water state dominated by green algae or a turbid-water state dominated by blue-green algae; once blue-green algae get established, they shade and thereby repel green algae, creating a self-perpetuating stable state. For systems with alternative stable states, one concept of stability depends on the number of alternative stable states: More stable systems are those with fewer stable states. Another concept of stability, Holling's resilience (*9*), describes the ease with which systems can switch between alternative stable states, with more stable systems having higher barriers to switching.

Owing to interactions among species, systems might fluctuate even in the absence of environmental perturbations (Fig. 1B). The resulting population dynamics are governed by "attractors" that can themselves be stable and hence regular (periodic), or can be unstable (chaotic) (*12*, *13*). The most familiar nonpoint attractor is a predator-prey stable limit cycle, in which the strong interactions between prey and predator generate perpetually oscillating densities (*14*). One measure of the stability of systems with nonpoint attractors is whether the attractor is chaotic. Another concept of stability that applies to either chaotic or nonchaotic systems depends on the amplitude of fluctuations, with more stable systems having lower-amplitude fluctuations of some aggregate measure of the system.

If the system has a single, stable equilibrium point, species densities will not fluctuate in the absence of environmental perturbations. Nonetheless, environmental perturbations may occur in the form of pulses or shocks that change species densities (Fig. 1C). If these pulse perturbations occur rarely, stability can be measured by the rate at which the system returns to equilibrium (*15*). If shocks occur frequently and stochastically, the impact of these shocks depends on community resistance (*5*), which can be measured by the variability in the change in combined densities, from one time point to the next, caused by repeated shocks. These two concepts of stability—the rate of return to equilibrium, and the change in combined densities in response to repeated shocks—together determine a third measure of stability: the overall system variability. For example, a more resistant system is knocked less by environmental shocks, and rapid return rates pull the system more quickly toward its equilibrium, both of which lead to lower overall community variability (*16*).

In addition to shocks, environmental perturbations may also cause permanent, "press" changes in demographic characteristics of species (*17*), such as decreasing intrinsic rates of increase. For example, Frost *et al.* (*18*) divided a lake with an impermeable membrane and then acidified one half, showing how the direct effect of acidification on planktonic species, and the interactions among them, changed the structure of the community. Press perturbations may change not only the equilibrium (*19*) but also, when severe enough, the dynamics around equilibrium (*20*) (Fig. 1D). A more stable system might be one whose combined species densities at equilibrium change more slowly when subjected to a press perturbation, or one that can sustain greater press perturbations before the dynamics undergo a qualitative change (e.g., one species goes extinct, or a point equilibrium bifurcates into a cyclic attractor).

Perturbations might also include the extinction of species (Fig. 1E) or the invasion of new species (Fig. 1F). When an extinction occurs, stability could be measured by the number of other species that go secondarily extinct, or by the compensatory change in combined densities of all species (*21*–*23*). When invasions occur, stability could be measured as the chance that an invader is successful, or the number of secondary extinctions it causes if it is successful (*24*).

This collection of stability concepts sets an empirical challenge. Before designing an empirical study, it is necessary to know enough about the dynamics of an ecosystem and the environmental perturbations that impinge upon it to select appropriate definitions of stability; there will often be several appropriate definitions. These concepts also identify key features—we will refer to them as mechanisms—that together dictate stability. These mechanisms involve the strength of interactions among species, the mode in which species interact (whether they are competitors, predators, mutualists, etc.) that gives the food-web topology, and the ways in which species experience different types of environmental perturbations. Because both species interactions and environmental perturbations can

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drive fluctuations in species densities, these must be sorted out and quantified to understand their mechanistic roles in diversity-stability relationships.

Review of Empirical Studies

We performed a survey of 52 studies giving 64 diversity-stability relationships (table S1); 48 of the 52 were published since 1990, reflecting the burgeoning interest in empirical tests of such relationships. Of the 52 studies, 37 directly manipulated diversity; the remaining 15 either used indirect manipulations of diversity or intentionally selected systems that differed in diversity. The most common definitions of stability were invasibility, variability, resistance, and return rates, making up 59 of 64 relationships; of these 59, the authors reported positive relationships in 41 cases (69%) and negative relationships in only 8 cases (14%), with no or ambiguous relationships for the remainder (table S2). Positive relationships were most commonly reported for studies on the success of invasive species (85%). Of the 18 studies measuring community variability, 72% showed positive relationships, consistent with the "consensus" view that greater diversity leads to less variable communities (*25*). Nonetheless, empirical studies have focused on only a subset of possible definitions of stability; more than half of the commonly used theoretical definitions have not been investigated experimentally to determine the role of diversity (table S2).

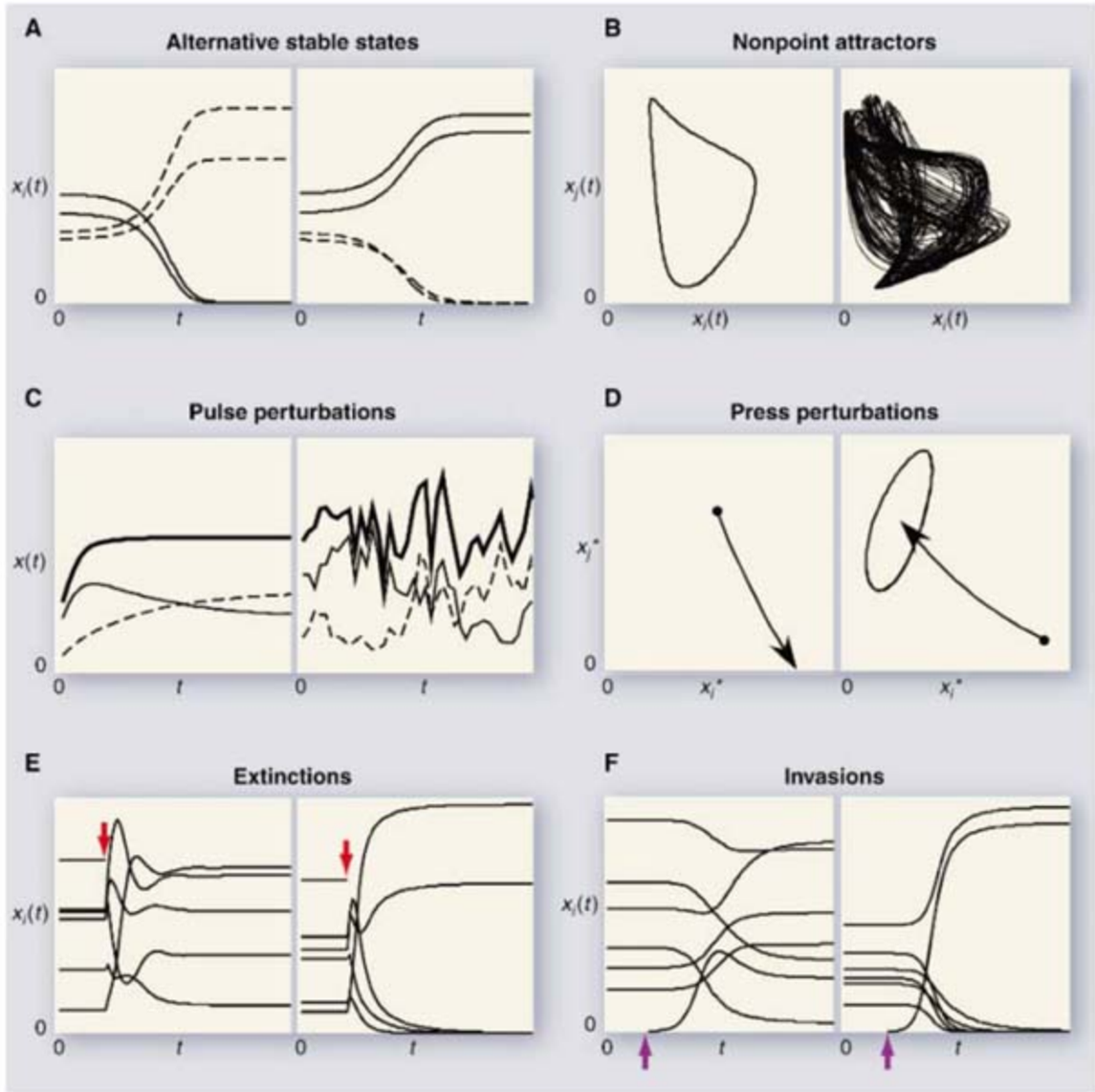

Fig. 1. Different types of stability, depending on the inherent dynamics of a system and the type of perturbation it experiences. (**A**) Alternative stable states, in which the initial densities of four species determine which species persist; pairs of alternatively persisting or nonpersisting species are shown with solid and dashed lines, respectively. (**B**) Nonpoint equilibria, illustrated by a stable and a chaotic attractor. (**C**) Pulse perturbations to systems with a stable equilibrium. The left panel shows the dynamics of a two-species system after a single pulse perturbation, with species densities shown by light and dashed lines, and combined densities shown by the heavy line. The right panel gives the same system with repeated (stochastic) pulse perturbations. (**D**) Press perturbations to systems with a stable equilibrium. The arrows trace the equilibrium densities of species *i* and *j* in a six-species ecosystem as the environment degrades (intrinsic rates of increase decline for all species). In the left panel, the equilibrium point collides with the unstable point at which species *j* goes extinct; in the right panel, the equilibrium point bifurcates into a stable nonpoint attractor. (**E**) Response of ecosystems to extinctions of the most common species (extinction marked by arrow). In the left panel, no other species went extinct; in the right panel, three additional species went extinct. (**F**) Response of ecosystems to invasion (invasion marked by arrow). In the left panel, the invading species persisted with the original six species; in the right panel, five of the original species went extinct. See fig. S2 for details.

A striking feature of the studies is how heterogeneous they are. Thirty-three studies investigated grassland or herbaceous plant communities, 9 investigated microbial communities, and 10 investigated other types of ecosystems. Forty-one studies included measurements on only a single trophic level; the remainder measured multiple trophic levels or included measurements that integrated over three or more trophic levels, such as microbial studies measuring CO_2 production. With this heterogeneous mix, it would be incautious to perform a meta-analysis to try to derive broad conclusions about diversity-stability relationships.

Another striking feature of the studies is how few rigorously investigate the mechanisms—species-species interactions, food-web topology, and the sensitivities of species to environmental perturbations—underlying reported diversity-stability relationships. Exceptions are some studies on invasibility. For example, Stachowicz *et al.* (*26*) showed that more-diverse intertidal communities leave less rocky surface exposed, thereby inhibiting invasive species, and Dukes (*27*) obtained a similar result for grassland communities; these studies thus show the role of species interactions in determining invasibility. Understanding the mechanisms underlying other types of stability is more difficult, especially those involving population dynamics (e.g., return rates and community variability). However, if the mechanisms underlying diversity-stability relationships are not identified, it is unclear whether an observed diversity-stability relationship can be generalized to any other system.

Our understanding of such mechanisms can be aided by statistically tying data to theoretical models. Although all empirical studies qualitatively compare their results to theory, too often mismatches between experiment and theory made it impossible for us to assess the experiments in the context of theoretical predictions (fig. S1). If we wish to assess empirical results in

the light of theory, it is not sufficient for theory to predict correctly whether the diversity-stability relationship is positive or negative; models could give the right prediction for the wrong reasons. Instead, theoretical models must be judged by their ability to capture the entire dynamics of the empirical system. For example, for a study focusing on stability measured by community variability, the test of the model is its ability to fit the dynamics of all species in the community in a statistically rigorous way. The process of model fitting requires the explicit identification and quantification of species interactions, as well as the response of individual species to environmental perturbations (*16*, *20*).

We know of no study on diversity and stability that explicitly fits a mechanistic model to data. But if we drop the requirement that the study focus on diversity, there are numerous studies on stability that fit models to data. For example, Wootton (*28*) tested the ability of a Markov chain model to predict the consequences of species extinctions on the densities of species remaining in intertidal communities; the success of this model relied on its ability to quantify the key interactions among species. As another example, Klug *et al.* (*29*) measured the responses of freshwater plankton to pulsed and press decreases in pH, determining both the sensitivity of species to the perturbation and how species-species interactions propagated the perturbation through the food web. Although these studies do not reveal the role of diversity, they suggest how the systems might change if different species were lost.

Review of Theory

There is a vast theoretical literature that is relevant to the relationship between diversity and stability (*6*). To order this literature, we used a single, simple model (Fig. 2). The use of a single model emphasizes that the same system may exhibit numerous diversity-stability relationships arising from different definitions of stability. It also shows that the same mechanisms can lead to different diversity-stability relationships. Our model considers only competitive interactions (one trophic level), although a version with two trophic levels (fig. S2) gives many similar relationships. Although this exercise is exactly the type of theory that is not useful for understanding real data from real systems, it is nonetheless valuable to hone our intuition and catalog numerous possible diversity-stability relationships.

Of 13 diversity-stability relationships that we computed for 13 definitions of stability, four were positive, six negative, and three nearly zero (Fig. 2, A to F). Furthermore, species-rich systems were more likely to show a greater range of diversity-stability relationships; the prevalence of systems with alternative stable states and nonpoint attractors increased with diversity (Fig. 2G). The patterns exhibited by the simple model are generally consistent with the broader theoretical literature (*6*), although some diversity-

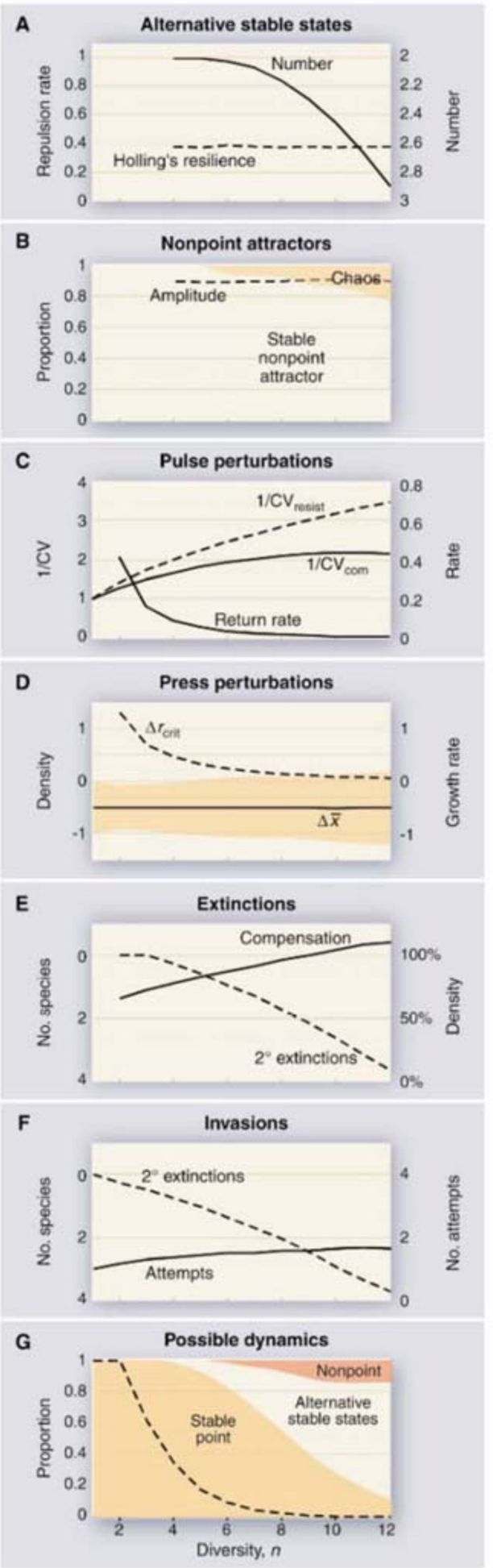

Fig. 2. Stability of randomly constructed competitive communities versus diversity n, portrayed so that positive diversity-stability relationships have positive slopes. (**A**) For systems with alternative stable states, the average number of stable states and Holling's resilience, measured by the rate at which population densities are repelled from the unstable stationary point between stable states. (**B**) For systems with nonpoint attractors, the prevalence of cyclic (white region) versus chaotic (orange region) attractors, and the amplitude of fluctuations in combined species densities, measured by the minimum divided by the maximum density (dashed line). (**C**) For systems with stable equilibria, the characteristic return rate, $1/CV_{resist}$, and $1/CV_{com}$, where CV_{resist} is the coefficient of variation in the change in abundance between samples, and CV_{com} is the coefficient of variation of the community density through time. (**D**) The change in mean combined densities, $\Delta\bar{x}$ (with 95% inclusion bounds given by the orange region), when all species experience a press perturbation that decreases intrinsic rates of increase. Δr_{crit} measures the magnitude of the press perturbation before the stable equilibrium bifurcates, creating either a cyclic nonpoint attractor or an attractor with one species extinct. (**E**) For systems with a stable equilibrium, the numbers of secondary (2°) extinctions caused by removing the most common species, and compensation (calculated as the increase in combined abundances of surviving species immediately after extinction relative to the abundance of the species that went extinct). (**F**) For systems with a stable equilibrium, the number of attempts before an introduced species successfully invaded, and the numbers of secondary extinctions caused by the invader. (**G**) For randomly constructed communities, prevalence of stable points, alternative stable states, and nonstationary attractors. The dashed line gives the proportion of randomly constructed communities that were feasible (i.e., had an equilibrium point with positive densities of all species), which is a requirement for the three types of dynamics. For each level of diversity n, 10,000 random communities were constructed. See fig. S2 for details.

stability relationships have not received sufficient attention to make general theoretical predictions with any confidence (table S2). Also, we caution that these patterns represent the averages of 10,000 randomly constructed communities; for any given model community, the diversity-stability pattern might differ (e.g., Fig. 2D).

Despite this picture of complexity, there are some generalities. For definitions of stability involving dynamics, species-species interactions (competition) tend to create negative diversity-stability relationships. Specifically, species-species interactions underlie the existence of alternative stable states (Fig. 2A) and nonpoint attractors (Fig. 2B). Similarly, species-species interactions cause the decrease in return rates with increasing diversity (Fig. 2C). Conversely, species-environment interactions underlie the positive diversity-stability relationship for resistance, $1/CV_{resist}$; when species respond differently to environmental variation, the variation in their combined, ecosystem-level response decreases with increasing diversity n, because the decreases in abundance of some species are counterbalanced by increases in others. In the parameterization of the model used for Fig. 2, the effect of species-environment interactions to increase community resistance dominates that of species-species interactions to decrease return rates, causing a positive diversity-stability relationship when stability is measured in terms of variability, $1/CV_{com}$. Nonetheless, the destabilizing effect of species-species interactions is seen in the decrease in $1/CV_{com}$ relative to $1/CV_{resist}$; in the absence of competition, these two measures would be the same.

Rather than generalities, the model more successfully reveals complications. For example, two measures of stability in response to the same perturbation can show opposite diversity-stability relationships. This is seen for perturbations caused by extinctions: After extinctions, species-rich communities are more likely to suffer secondary extinctions (negative diversity-stability relationship) yet also show greater compensation (positive relationship). In the model, compensation is so strong that despite secondary extinctions, combined species abundances on average increase when the most common species goes extinct from ecosystems with 12 species. Similarly, species-rich communities are more likely to repel invaders (positive diversity-stability relationship), yet if the invader is successful it is likely to cause more secondary extinctions (negative relationship).

As another complication, the same mechanism can have different effects. For example, competition generally destabilizes dynamics, increasing the likelihood of alternative stable states and nonpoint attractors, and decreasing return rates to a stable equilibrium point. Nonetheless, for definitions of stability not involving dynamics, competition is not destabilizing. For press perturbations (Fig. 2D), the average decrease in abundance is 0.5, the same as would occur if there were no competition. For compensation after extinctions, competition is stabilizing, because in the absence of competition, no compensation would occur. In these examples, competition is destabilizing, neutral, and stabilizing, respectively. These complications underscore the need to understand the mechanisms underlying diversity-stability relationships.

Finally, we return to the empirical studies and compare them with the broad theoretical patterns. Of the four types of stability most heavily represented (59 of 64 relationships), two (invasibility and resistance) generally give theoretical diversity-stability relationships that are positive, and a third (variability) will give a positive relationship when the effect of diversity on resistance is large; together, these make up 50 of 64 relationships. This suggests that the preponderance of empirical studies showing positive relationships (43 of 64, table S2) do so because they use definitions of stability that are likely to show positive relationships. Nonetheless, theory generally predicts negative diversity-stability relationships for stability measured as return rates, yet eight of the nine empirical studies that used this measure reported a positive or no relationship (table S2). Given the frequent mismatches between empirical studies and theory, we think it is difficult to draw any strong conclusions from the empirical studies. This reemphasizes the need to statistically fit models to data.

Which Diversity-Stability Relationships?

With the many definitions of stability, we must ask which definitions are most relevant for applied problems surrounding the loss of biodiversity. The pressing questions of applied ecology involve human drivers, including climate change, nutrient input, toxins, invasive species, overexploitation of biological resources, and land use change (*30*). These drivers may interact; for example, climate change and species interactions have altered fire regimes in Alaskan boreal forest, thereby altering the dynamics of the spatial mosaic of land cover (*31*). Furthermore, many changes are occurring at broad spatial scales across a landscape that is increasingly divided into small, relatively homogeneous fragments greatly different from the former, contiguous whole (*30*).

All of the definitions of stability we have described are relevant to at least several applied problems (table S3). Nonetheless, stability in the face of press perturbations is often central, because many human drivers change hydrology, biogeochemical inputs, or habitat characteristics that alter population growth rates, biotic interactions, biomass production, and numerous other processes that affect how an ecosystem functions. Some of these press perturbations will lead to ecological surprises as a result of unexpectedly extensive or irreversible changes in some processes or in ecosystem structure (*32*). The Millennium Ecosystem Assessment (*30*) concluded that "there is established but incomplete evidence that changes being made in ecosystems are increasing the likelihood of nonlinear changes (including accelerating, abrupt, and potentially irreversible changes) with important consequences for human well-being" (p. 11).

Increasing the relevance of empirical studies for applied problems argues for increasing the range of definitions of stability. It also highlights the interactions among multiple factors affecting stability. In much of the literature on diversity and stability, diversity is treated as an independent variable, with experiments designed to test for the effects of diversity "per se" by selecting species randomly from a species pool. However, diversity is unlikely to change in isolation from other drivers affecting ecosystem stability, and in fact these other drivers will likely be the main causes of loss of diversity. For example, land use change has a direct effect on ecosystem production, respiration, and carbon storage but also changes the diversity of plants and consumers, leading to further changes in carbon budgets (*33*). Thus, diversity is not a primary driver, but it might be a secondary driver. A key consideration is that if anthropogenic change decreases diversity, it will likely do so in a nonrandom way, as specific species are encouraged or eliminated by human action. In this case, the effects of loss of diversity cannot be disentangled from the effects of changing species composition (*34*), making the secondary effect of diversity on production understandable only in the context of the primary driver changing the ecosystem.

Recommendations

The relationship between diversity and stability has interested ecologists since the inception of the discipline (*35*), and the absence of a resolution reflects the complexity of the problem. Much of the complexity derives from the multiplicity of diversity-stability relationships, depending on the definitions of diversity and stability and on the context in which an ecosystem is perturbed. We cannot expect a general conclusion about the diversity-stability relationship, and simply increasing the number of studies on different ecosystems will not generate one.

Rather than search for generalities in patterns of diversity-stability relationships, we recommend investigating mechanisms. A given diversity-stability relationship may be driven by multiple mechanisms, and the same mechanisms may evoke different diversity-stability relationships depending on the definitions of diversity and stability. We need more studies revealing exactly what these mechanisms are. This requires models joined to empirical studies that can reproduce, in a statistically robust way, not only a diversity-stability relationship but also the dynamics exhibited by a system.

Several definitions of stability—in particular, stability against press perturbations—have received relatively little attention. Nonetheless, these definitions of stability are key to understanding emerging global challenges. Diversity is

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INTRODUCTION

MANY VOICES, ONE MESSAGE

KATRIN SCHÄFER AND YUN YING WILL PROBABLY NEVER CROSS PATHS. BUT IF the 48-year-old biological anthropologist at the University of Vienna, Austria, and the 82-year-old physics educator at Southeast University in Nanjing, China, chanced to meet at a conference on global undergraduate education in the STEM (science, technology, engineering, and mathematics) fields, they might recognize each other as kindred spirits.

Both Schäfer and Yun are convinced that their students—native speakers of German and Chinese, respectively—need to possess a solid grasp of English to succeed as scientists or even lay claim to being scientifically literate citizens of the world. The hegemony of English is just one of many forces shaping undergraduate STEM education. This special issue looks at the topic by focusing on the lives of Schäfer, Yun, and 10 other faculty members in a dozen countries on six continents. The group is meant to be representative of scientists teaching large numbers of undergraduates around the world. The list is skewed toward the most industrialized countries but also includes those in which the scientific infrastructure is developing rapidly. An accompanying map presents some basic information about higher education in each country.

Despite the vast differences in the makeup of their students, the policies that govern higher education, and the cultural and economic factors that shape the profession, these scientists speak in surprisingly similar voices. In story after story, they point to lagging interest and poor preparation in science among students, insufficient resources, heavy professional burdens, and antiscience attitudes in society at large. Yet there are patches of light among those dark clouds. Each faculty member has managed to bring science to life for students in exciting and innovative ways. They are also engaged in myriad activities outside the classroom—from informal science education to election campaigns—aimed at spreading knowledge and the values of scientific thinking.

For an additional perspective, *Science* invited three distinguished educators to explore the issues facing undergraduate STEM education. Excerpts of their comments appear in this issue; the complete discussion is available at www.sciencemag.org/sciext/undergrad_education07. This issue also marks the debut of the Signal Transduction Knowledge Environment Journal Club, as well as three Teaching Resources.

We hope that you'll find the entire package compelling enough to alter your own worldview of undergraduate education. If it does, please let us know at www.sciencemag.org/sciext/eletters.

–JEFFREY MERVIS

THE WORLD OF Undergraduate Education

CONTENTS

See also related Editorial page 17; Science Careers and Science's STKE material on page 11 or at www.sciencemag.org/sciext/undergrad_education07

Science

Keeping Score

HIGHER EDUCATION IS A VAST AND COMPLEX ENTERPRISE in the industrial world and a rapidly growing sector for many developing countries. Although many groups try to keep tabs on that enterprise, data tend to be spotty and not always comparable. That's even more the case for statistics relating to STEM (science, technology, engineering, and mathematics) education.

Still, it is possible to quantify some aspects of the system. These pages offer basic information on five important components of STEM education, with material supplied by national governments and international bodies such as the Organisation for Economic Co-operation and Development. The data highlight the considerable challenges facing reformers in countries with large populations and those with small fractions of the student population entering STEM fields.

MAP: K. KRAUSE/*SCIENCE*

China

Population
▶ **1.31 billion**

Undergraduate degree-granting institutions
▶ **701**

18- to 22-year-olds pursuing postsecondary degrees ▶ **21%**

South Korea

Population
▶ **48.3 million**

Undergraduate degree-granting institutions
▶ **201**

18- to 21-year-olds pursuing postsecondary degrees ▶ **82.1%**

Japan

Population
▶ **127.7 million**

Undergraduate degree-granting institutions
▶ **726**

18- to 24-year-olds pursuing postsecondary degrees ▶ **47.3%**

Australia

Population
▶ **20.4 million**

Undergraduate degree-granting institutions
▶ **44**

18- to 24-year-olds pursuing postsecondary degrees ▶ **19.7%**

United States

Population
▶ **300 million**

Undergraduate degree-granting institutions
▶ **2500**

18- to 24-year-olds pursuing postsecondary degrees ▶ **64%**

Brazil

Population
▶ **189 million**

Undergraduate degree-granting institutions
▶ **2398**

18- to 24-year-olds pursuing postsecondary degrees ▶ **11.2%**

Native skills. Kath Handasyde (*left*) shows student Natalie Briscoe how to attach a radio collar using a stuffed wallaby.

AUSTRALIA

'A Crisis in Student Quantity and Quality'

Kath Handasyde enlists native species, assertive Americans, and anything else on hand to rekindle a passion for science among undergrads

MELBOURNE, AUSTRALIA—Tromping down an academic hallway with her flyaway shock of reddish-gold hair and a thin braid shooting out from behind one ear, Kath Handasyde looks like she's just wandered in from the Australian bush. In fact, she has. But the University of Melbourne (UM) ecologist, who specializes in Australia's endangered native mammals—particularly the egg-laying duck-billed platypus—is already hunting for another of Australia's endangered native species: undergraduate science majors.

She finds them clustered around a low table in the Zoology Department tearoom. Devi Stuart-Fox, their instructor, lets the undergraduates do the talking. "We're using guppies to measure the evolutionary tradeoff between behaviors for attracting mates versus avoiding predators," says Danial Hunter, a third-year student. They're hoping the project will result in a peer-reviewed publication.

Back in her office, Handasyde, a compact, 48-year-old, fast-talking ball of energy, raves about her students. "In a good year, up to half of the undergraduate projects produce a publication," she says, "and many students even publish two papers before they graduate." That impressive track record is partly due to what Handasyde calls "hands-on, research-focused teaching," including exposing all zoology majors to the trials and tribulations of grant writing. "It was extremely useful," says one of her students, Natalie Briscoe, who successfully persuaded a panel of classmates to fund an imaginary research project—before winning a genuine $5500 government award for a developmental study of caterpillars.

But Handasyde and her colleagues worry that such high-achieving science undergraduates are becoming increasingly rare. "We're facing a crisis," says Peter Rathjen, the UM dean of sciences, "both in terms of quantity and quality of students." The overall fraction of Australian undergraduates choosing science-related fields has held steady at about 20%, but growth in specialized applied fields, such as information technology, has "masked" a steady decline in the basic sciences, he says.

That decline has had a corresponding effect on the number of faculty positions because Australian universities, including UM, receive government funding on a per-student basis. "We're facing a serious challenge," says Rathjen. Mathematics has taken the biggest hit, with a 30% decline in faculty slots across the country over the past decade.

Growing up in a rural area, Handasyde decided at age 6 to become a zoologist. She tries to emulate the "passion and excitement" that she experienced 30 years ago as an undergraduate in the same department. "The classes were smaller, and staff were less loaded with the huge diversity of modern tasks that we undertake now," she says.

But that happy story doesn't seem to hold true elsewhere. To keep their classrooms filled, many science departments have needed to lower entrance requirements. "In effect," says Rathjen, "universities are taking in students to study science who do not have the preparation, and possibly ability, to complete courses of proper rigor." He says that high school students have been allowed to drift away from taking challenging courses such as calculus, and teachers lack incentives to upgrade their knowledge.

But it isn't all gloom and doom. One positive trend in the undergraduate ranks, says Handasyde, is the massive influx of overseas students in the past 5 years. Most come to Australia from newly affluent eastern Asia with their sights set on careers in business, biotechnology, engineering, and medicine. The added ethnic diversity—a quarter of Australian undergrads now hail from abroad—"really opens the world for our students," she says.

About 6% of the overseas students come from the United States, a trend that brings a smile to Handasyde's face. "What we all love about the American students is how much more assertive they are in the classroom than us Aussies," she says. She avoids using the moniker "good-natured loudmouths"—a common term here—but her point is clear. "They spark conversations [in classes] where teachers usually struggle to get students to interact."

At the same time, the increasing diversity hasn't corrected a serious underrepresentation on campuses of indigenous people. Although as many as 5% of Australians are indigenous, they make up only 1% of the student body. And science is near the bottom of their list of majors, says Ian Anderson, director of UM's Centre for Health and Society. Poor preparation is one reason, he says, along with a lack of indigenous leaders in academia. Handasyde agrees. There is no easy fix, she says, but "what we badly need are more success stories."

One piece of good news arrived this spring with the announcement of a budget windfall. The government is setting aside an extra $4 billion next year as an endowment, with the interest going toward university infrastructure upgrades. That will be a boon to science, says Rathjen, "because our teaching and lab facilities are stuck in the 1960s and '70s." The government is also revising its formula for funding universities, with a significant boost for scientific courses, particularly those involving labs. "I can't believe it's taken this long," says Rathjen, "but we're finally coming around to seeing that our future depends on our scientists."

–JOHN BOHANNON

CREDIT: J. BOHANNON/SCIENCE

UNITED STATES

'This Is the Front Line ... Where I Can Really Make a Difference'

Lisa Park and her colleagues take on creationism and other antiscientific attitudes in the classroom—and in the voting booth

AKRON, OHIO—Lisa Park's introductory physical geology class at the University of Akron fills from the front and the back of the room simultaneously. Some students hustle into the front seats and chat eagerly with Park, while others drift into the very last row, leaving empty seats in front of them.

When Park announces that the upcoming final exam will cover material from the whole term, rather than just the last few weeks, the students in the back row aren't happy. "This is the hardest class!" hisses one student, blonde hair wet but eye makeup firmly in place at 9:15 a.m. Her neighbor, whose head is down on the desk, doesn't appear to hear.

By the end of the hour, however, even those in the back row have bestirred themselves to do a small-group exercise with giant plastic relief maps. The entire class can now explain that mountains form where tectonic plates converge. Score one for scientific literacy.

"I've been at a private liberal arts school, a big research I university, and here. This is the front lines," says Park about the University of Akron, an open-enrollment university in a northeastern Ohio city that's hoping to replace its lost manufacturing base with polymer science and biotechnology. A sizable fraction of students at the university are the first in their families to go to college, and a third don't make it beyond the first year, says geology professor David McConnell, co-author with Park and others of a new introductory earth science textbook. "This is their leg up to get somewhere," says Park. "This is where I can really make a difference."

Park, 41, can identify with them. She grew up in a blue-collar suburb of Cleveland, the daughter of a NASA engineer and a teacher. "I tell people I couldn't be elitist even if I tried. I'm from Parma, Ohio," says Park. She received her B.A. from the nearby College of Wooster and headed west—to the University of Arizona—for her Ph.D. before returning to the area in 1995 to join the faculty of the University of Akron.

With relatively few of their students aiming to become research scientists, Park and her colleagues are gearing their efforts toward scientific literacy. "The goal is that 5 years from now, they can process information on, say, global warming in a reasonable way," says McConnell. Adds Park, "We need to educate [students] as citizens."

The envelope, please. Geologist Lisa Park looks for fossils that can serve as paleoclimatic markers when she's not helping to create scientifically literate students.

To engage their students, Park, McConnell, and others try to make science relevant with "inquiry-based" exercises like the one with the relief map. During another of Park's classes, for example, students brought in bottled and tap water. Sophomore Sarah Rolan, 24, recalls that a chemical analysis found that a leading brand of water had a pH of only 4.5. Says Park: "You've got to make it relevant or you lose them. ... Their eyes glaze over when you talk about groundwater. But water they personally drink? They were totally into it."

Having a diverse cross section of students also means that Park and her colleagues often confront mainstream attitudes toward science, including creationism. In recent years, Park has seen a tide of creationism rising both on campus and off. "We teach almost literally in the shadow of The Chapel," notes anthropology instructor John Reeves, referring to an evangelical megachurch on the edge of the university's urban campus.

Creationist speakers visit the campus fairly regularly, sponsored by religious groups or a "critical thinkers club." In her geology classes, Park explicitly debunks the idea that the biblical flood formed the Grand Canyon. Many of her students have only a sketchy background in evolution. "My high school biology teacher only went over it for a couple of days—didn't want to get into it," says freshman accounting major Kacy Grogg, 19. Junior Brandon Behnfeldt, 21, who plans to be a biology teacher, allows that the scientific age of Earth is more reliable than the biblical one. But when it comes to evolution, he says, "I hold with intelligent design." If he takes Park's paleontology course, he'll hear an entire lecture that skewers intelligent design arguments.

Last fall, Park and her colleague, biology professor Stephen Weeks, worked nonstop to elect a pro-science candidate to the Ohio Board of Education. "I could not stand by and do nothing," says Park. She analyzed local polling data that were later used to deploy volunteers on voting day. "I realized, this is paleoecology. I have two species, Democrat and Republican, and I'm looking at their site distribution."

Such hard work involves tradeoffs, however: Park and Weeks each missed a January deadline for submitting research proposals to the National Science Foundation (NSF). "To me, fighting for evolution is part of my job," says Weeks. "But the system is not set up to benefit those who make this kind of move."

Park has been funded by NSF, despite a low success rate in paleobiology, and by other sources—enough to support research by a small group of undergraduate and master's students. "One thing that got me into paleontology is just handling the fossils," says Park. Her goal is "to keep that wonderment, that discovery, alive."

To judge by senior Melissa Kindle, Park's approach is working. "I want to do what she does," says Kindle, confidently screening core samples in the sink. "I want to be a paleontologist." **–ELIZABETH CULOTTA**

CREDIT: E. CULOTTA/SCIENCE

UNITED KINGDOM

'Much of What We Were Doing Didn't Work'

Derek Raine sees integrated sciences as a potential savior for disciplines facing declining student interest and a dwindling number of departments

LEICESTER, U.K.—Teaching physical sciences at a British university can be a precarious business these days. Just ask Derek Raine, a physicist at the University of Leicester who has worked diligently to boost both enrollment and student achievement at this midsize physics department.

Raine has been in the forefront of efforts to introduce innovative teaching strategies and a new degree program known as Integrated Sciences. Yet despite the country's rapidly growing university system, applications for some subjects, including physics and chemistry, have been stagnant. "Big departments are sucking in more [students]," says Raine. As a result, 21 physics departments have closed over the past decade, and more than half of all U.K. universities no longer offer undergraduate chemistry courses. Even the likes of the University of Leicester are struggling to fill their courses.

Physics and chemistry are caught in a vicious circle. High demand for graduates from high-tech industry and the financial sector has meant fewer students going into teaching. That depleted teaching corps—some 25% of U.K. high schools, for example, now have no specialist physics teacher—could mean fewer inspiring teachers, says Raine, which leads to fewer students selecting physics or chemistry at university. (At age 16, U.K. pupils choose just three or four subjects to continue through to graduation at age 18, and they study their major almost exclusively for 3 years at university.)

This dire situation has developed despite a 42% growth in the U.K.'s undergraduate population between 1995 and 2005 and a government campaign to get at least one-half of all young people to attend university. But some subjects have lost ground: The total share studying physical sciences has dropped by nearly one percentage point since 1998, to 3.7%.

Hence Raine's struggle to boost Leicester's undergraduate physics program. He himself trained at Cambridge University and did postdoctoral work at Oxford in quantum field theory and astrophysics that branched into biophysics. His experience teaching at the University of Leicester made him "see how much of what we were doing didn't work," he says, especially for the average or struggling student. His search for a better answer led him to problem-based learning (PBL), an approach often used in medical schools and pioneered at Canada's McMaster University in the 1970s.

Currying curiosity. Derek Raine says students "are hooked into working hard" by courses they find interesting.

Working in teams, students are given a real-world problem to research, solve, and then explicate to the class. One exercise casts students as the crew of a cargo plane that has crashed on a desert island and asks them to construct some sort of beacon to communicate their position. Such problem solving "is what we do every day as researchers," says Sarah Symons, head of a project to develop PBL at Leicester. "I can't see why it's not obvious to everyone."

Today, after 7 years of work, about 25% of Leicester's physics courses are taught using PBL, and three-quarters of staff members are involved in some way. Leicester's chemistry department is developing a PBL course, and a few other U.K. universities are altering their curricula. Although studies of the effectiveness of PBL have produced contradictory results, some suggest that students retain their knowledge longer and are better at applying it to real-world situations.

The U.K. government is also belatedly tackling the problem. The lack of action on physics education is a "big failure" of Tony Blair's government, charges Peter Cotgreave of the Campaign for Science and Engineering in London. But after some high-profile department closures (*Science*, 4 February 2005, p. 668, and 1 December 2006, p. 1363), the government last year put up $150 million over 3 years to help finance expensive lab-based courses and gave grants to physics, chemistry, engineering, and mathematics societies to stimulate demand for and improve curricula. Raine is involved in one such project being run by the Institute of Physics (IOP) in London to create a multidisciplinary degree with a healthy dose of physics. "Derek's always up for something new," says IOP's Philip Diamond.

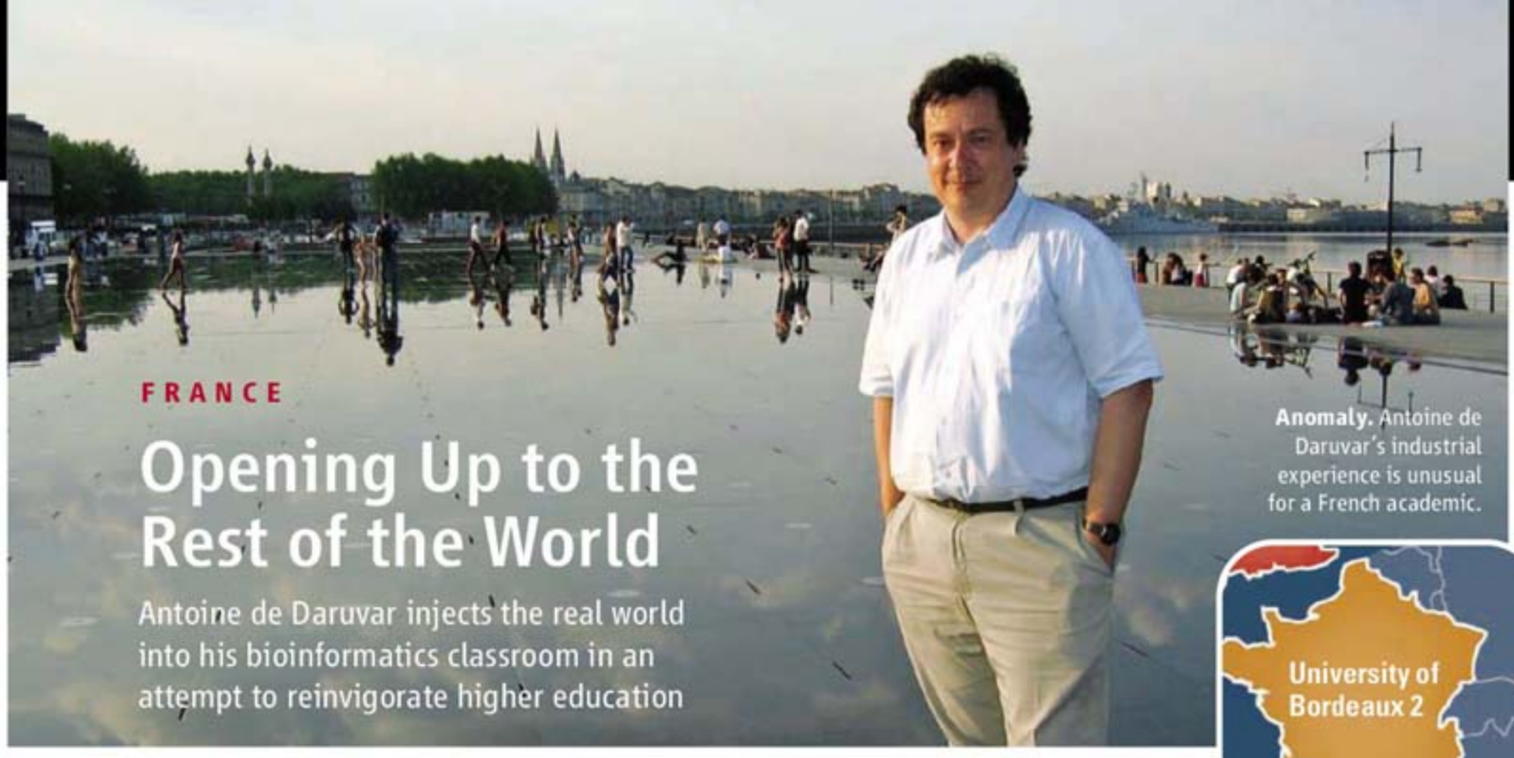
Raine actually began developing such a degree 5 years ago in an attempt to expand science enrollment at Leicester. His Integrated Sciences course has been running for 3 years now, using PBL extensively. The curriculum asks students how they might have built the pyramids of Egypt and used them for astronomy, and then moves on to space science, nanotechnology, biomechanics, and quantum teleportation, among other subjects. All of these topics are presented in PBL scenarios such as mock court cases, film productions, and preparations for the 2012 London Olympics. "You can ask any question, no matter how stupid," says second-year undergraduate Ben Watson.

Undergraduates aren't yet flocking to pursue the Integrated Sciences degree, however. This year's first graduating class numbers only six students, and in a typical year, fewer than 10 enter the program. "Students are wary of something new," says course administrator Alex Mack, and sometimes unsure of what it prepares them to do. Raine thinks it's ideal training for interdisciplinary research, science-based industry, or teaching. Raine is hoping that IOP's decision to sponsor similar degrees at three other universities—Surrey, University of East Anglia, and London South Bank—will create a "brand" that will boost enrollment.

"Our vision is that all science students should initially come in studying Integrated Sciences," Raine says. "Not everyone is going to be a string theorist."

–DANIEL CLERY

CREDIT: COURTESY OF DEREK RAINE



FRANCE

Opening Up to the Rest of the World

Antoine de Daruvar injects the real world into his bioinformatics classroom in an attempt to reinvigorate higher education

Anomaly. Antoine de Daruvar's industrial experience is unusual for a French academic.

BORDEAUX, FRANCE—It's the day of reckoning in Antoine de Daruvar's bioinformatics class at the Université Victor Segalen Bordeaux 2—time for his students to show what they have learned the past semester. Their assignment was to write a computer application to help biologists search genetic databases, and Daruvar is eager to test-drive the results.

"Hmmm … is that image free of copyrights?" he asks a duo who have embellished their start page with a picture of a magnifying glass. "Mission accomplished," he beams at the authors of a nearly flawless piece of software.

Daruvar, director of the Centre for Bioinformatics of Bordeaux (CBIB), is a new kind of professor on the French academic landscape. With roots and continuing ties to the business world—"an anomaly" on French campuses, says microbiologist and colleague Alain Blanchard—he's well-equipped to promote technology transfer, a weak link in the French economy. "The university doesn't have enough people like him," says Béatrice Chassaing of the Regional Council of Aquitaine, which is supporting CBIB. Chassaing says she's also impressed with Daruvar's "dynamism and enthusiasm."

Daruvar, 44, strongly agrees that French universities should open up to the rest of the world. He encourages his students to do internships or projects abroad, and he hopes to create a master's degree in functional genomics, taught entirely in English. Opponents say such programs will deal the coup de grâce to French as a scientific language.

His own atypical background helped shape his conviction that French higher education needs to make some drastic changes. After obtaining a master's degree in informatics in 1987, Daruvar worked as an industrial software engineer and a bioinformaticist at the European Molecular Biology Laboratory in Heidelberg, Germany, before joining a bioinformatics start-up company there. He followed his neuroscientist wife when she got a job in Bordeaux, where he was asked to set up bioinformatics training within the biology curriculum. That work led to a professorship, which represented a 50% salary cut but lifetime job security as a civil servant.

With a dozen employees, CBIB provides software support to the university's life scientists, conducts joint research—for instance, in comparative genomics studies of bacteria—and does some independent work in informatics. But it's the teaching that Daruvar loves most, especially when he can infect his students with his own fascination for the art of writing software. (Judging from a few glazed looks, not all of his pupils have caught the bug yet.) He's perplexed by other aspects of academic life, however, in particular the lack of institutional autonomy.

For instance, prospective faculty members must be "qualified" by peer panels at the National Council of Universities in Paris. Those panels meet only once a year, creating delays. Daruvar's career path and shorter publication list did not bother the university, but they raised questions for the panel. Daruvar says the system is "absurd" and "incomprehensible to foreign scientists" who are repelled by such seemingly arbitrary hurdles.

Paris's power extends to many other areas of academic life and, according to Daruvar and others, stifles innovation. The education ministry requires every professor and assistant professor to teach 192 hours a year, regardless of other duties. At many universities, ministry-imposed budgets for everything from housing to maintenance make it almost impossible for universities to set new priorities, Daruvar says. (Bordeaux 2 was one of the first to negotiate a lump-sum budget recently.)

These issues have been debated in France for many years, and several governments have tried reforms—only to pull back in the face of protests from unions and students. The new government, too, has proposed giving universities more autonomy, and Daruvar believes it may have a better chance to succeed as the groundswell for reform grows. To wit, he says opposition to his plan for teaching a master's program in English is softening. The university and the ministry understand that France needs more foreign students, he says, and French students realize that English is crucial for their career perspectives. Besides, he argues, "it's too late" to rescue scientific French.

A few things are unlikely to change, however. Open enrollments and low tuitions mean that many students enter the university without knowing what they want to do and lacking motivation. As a result, more than 25% drop out in the first 2 years. "That's a big waste," Daruvar says, and a demoralizing experience for the dropouts.

Daruvar doesn't advocate changing those policies—"the universities have to remain open to all," he says, and indeed, the new government has said it won't touch the twin hot-button issues. But he would like to see students receive much more guidance when choosing a field and a university. Once students realize that the ivory tower isn't their only career option, he reasons, perhaps it will be easier to keep them on campus.

–MARTIN ENSERINK

CREDIT: M. ENSERINK/SCIENCE

BRAZIL

'I Do Not Make a Distinction Between Teaching and Research'

Antônio Carlos Pavão combines the ideal with the practical to bring science to the masses and create the next generation of scientists

RECIFE, BRAZIL—Leon Trotsky peers down on visitors to the tiny 3-by-5-meter space that passes for the office of Antônio Carlos Pavão at the Federal University of Pernambuco (UFPE) here in northeastern Brazil. The tenured professor of theoretical chemistry finds the concept of permanent revolution espoused by the bearded Bolshevik to be an apt metaphor for his efforts to reform science education. "The revolution does not proceed stage-wise but permanently," he points out. "The same applies to teaching."

Equally graying and bearded, the 56-year-old Pavão manages to incorporate the utopian view of the martyred Marxist without losing touch with reality. He's the only professor in the department who shares his office space with a quartet of graduate students, for example—but he doesn't lend out any of his cherished books. And on larger issues, he's adept at the art of the possible. As chair of a national panel to evaluate junior high science textbooks, he first argued that none deserved a seal of approval from the Education Ministry. "All the books have flaws," he explained.

In the end, he agreed that 13 of the 23 series warranted a place on the ministry's list, giving them a shot at a $335 million market reaching 31 million pupils. But he had made his point: Glossy pictures and hands-on demonstrations are no substitute for teachers, like himself, who are devoted to turning students into practicing researchers and who know how to find value from the most mundane resources. "What school in Brazil has no ants in the yard?" asks Pavão, who as a graduate student once sent São Paulo secondary students spinning in opposite directions to help them understand the structure of the atom.

Most science teachers are far from being that resourceful. The life of a Brazilian secondary school teacher is not easy, thanks to low wages, poor teaching conditions, and mounting violence. The resulting teacher shortage is particularly serious in science, technology, engineering, and mathematics: From 1990 to 2001, for example, only 7200 students earned a degree to teach physics despite the estimated 55,000 jobs available in the nation's schools.

With the corner druggist sometimes teaching physics in understaffed public high schools, it's no surprise that Brazilian 15-year-olds ranked last among 41 countries in mathematics and 40th in science in 2003 on an international assessment involving 15-year-old students. It also helps explain why up to 40% of students entering UFPE's Center for Exact and Natural Sciences, where Pavão teaches, drop out after the first semester; in other Brazilian federal universities, the number is as high as 75%. "Mathematics is the knot," Pavão explains, although he says students are also put off by the current university practice of lumping together all science, engineering, and mathematics students in courses devoid of labs and actual research.

Two years ago, Pavão and his colleagues began an experiment to circumvent the students' lack of preparation. They added a third phase to the traditional two-step admission process, which includes a massive multiple choice test followed by handwritten exams. UFPE now accepts three times more candidates than it has room for and offers them a series of one-semester, pre-entry science courses. Those who pass the closing

RUSSIA

'The Teacher Is Still the Central Figure'

Irina Sukovataya taps into software and the resources of a new megauniversity to help physics students

KRASNOYARSK, RUSSIA—In a small laboratory, first-year physics students are running electric current through a diode. Although the actual diode is in another room, its depiction on a computer screen allows students to work at their own pace, spares the equipment, eliminates accidents, and offers both students and teachers greater flexibility in what is typically a heavy work schedule. Biophysicist Irina Sukovataya devised this and similar software for her physics classes at Siberian Federal University (SFU)—and potentially for thousands of other students from across the vast region who can't travel here. "The software allows for an individual trajectory of study. A student can begin at any stage he likes," says Sukovataya.

In this country of 11 time zones, a university that is east of the Ural Mountains can be easily forgotten when almost all the people and money are to the west. SFU, less than 1 year old, is one of the country's four new megauniversities. Together with those in Moscow, St. Petersburg, and Rostov-on-Don—and a fifth planned for Vladivostok—the megauniversities represent the government's latest attempt to reward innovation and restore quality in higher education across the nation.

That initiative is part of a slew of Western-style reforms to Russian higher education that include creating two elite business schools and replacing the current 5-year degree with a more compact 4-year system in line with the Bologna process. SFU is due to receive $386 million by 2010, about eight times the combined budget of the four institutions that were merged into SFU. Refurbishing laboratories is a high priority, along with offering higher salaries and stipends to attract and retain faculty members.

So far, the 35-year-old Sukovataya is ahead of the curve. A graduate of Krasnoyarsk State University, now part of SFU, Sukovataya did graduate work at the Russian Academy of Sciences' (RAS) Institute of Biophysics here. In 2001, she joined the faculty of Krasnoyarsk State Technical University, also now a part of SFU.

Sukovataya has twice won an annual scholarship for excellence in teaching and research, along with a stipend that far exceeds her monthly salary. Her new teaching materials and automated methods of instruction are all the more

Science for all. Antônio Pavão (in orange T-shirt) plays with visitors at the science museum in Recife.

exams are allowed to enter the regular undergraduate program, which, like all Brazilian public universities, is free.

In his general chemistry classroom, Pavão puts on quite a show. He'll place a tomato on the table and ask why it's red. Or he'll grab a soccer ball from his briefcase and point out how well its pentagons and hexagons fit in a round surface, a seamless introduction to a fullerene molecule made up of 60 carbon atoms and an explanation of chemical bonds. "Better and more experienced professors should be teaching undergrad courses to freshmen and sophomores," he argues, aware that his advice is not usually followed.

His goal, he declares, is to explain the structure of matter. "I do not make a distinction between teaching and research. There is a fascination among students for advanced subjects. I take advantage of it," says Pavão. "At the end of the day, I think I end up helping them overcome their shortcomings and understand the need to study and learn more about those 'boring things' in mathematics, physics, etc."

His colleagues say that his work in both realms is top-notch. "Pavão is an original theoretical chemist, with very wide-ranging interests," says Nobelist Roald Hoffmann of Cornell University, whose picture hangs alongside the Trotsky poster. "Pavão's command of theoretical formalism and attention to experimental detail," Hoffmann explains, makes for "an appealing combination."

When not at UFPE teaching and doing research, Pavão directs Espaço Ciência, a state-owned, open-air science museum squatted in a semiabandoned exhibition pavilion between the cities of Recife and Olinda. Some of its yearly 80,000 visitors—mostly teachers and pupils—later attend summer hands-on courses at UFPE, another of Pavão's multiple activities. Those taking a 2-week course on "Kitchen Chemistry," for example, might employ a $300,000 spectrometer trying to solve the structural mystery of stale butter.

The initiative is part of a network set up by faculty members at 10 federal universities on a slim budget, and his ever-active mind sees it as a model. "UFPE has about 50 departments," he says. "If all engage in pilot projects such as ours, we could do 50 times more." In the meantime, Trotsky's looming presence reminds Pavão that providing every child in Brazil with access to the wonders of science requires a blend of utopian dreams and the patience and vigilance of a disciplined activist.

–MARCELO LEITE

Marcelo Leite is a writer in São Paolo.

remarkable because they arose in an academic environment still dominated by Soviet-era thinking. "We developed these materials because they are convenient and helpful, not because there was some kind of directive from the Education and Science Ministry," she says. "We began earlier, much earlier."

The diode software, developed at the multimedia lab run by her husband, Aleksei, a specialist in quantum electronics, gives students the option of studying when and where they want. That's a radical concept for those used to adhering to a rigid path laid down by federal officials. "In fact, when I see students with a very high level of ability, I try to direct them to study on their own, at home," says Sukovataya.

But that's easier said than done. Aleksandra Altukhova, whose father is a physicist, says that although a computer can replicate a piece of lab equipment, it cannot replicate a teacher. Besides, she confesses, it is more difficult to work independently, and Sukovataya can be persuaded to give hints not forthcoming from the software. The two have worked out a compromise, Sukovataya says: "I told her that she could come in once per month, not every week."

Similar software is also useful for distance learning. Students at technical schools across Siberia have used it to perform experiments online and even to direct robots, and the university eventually hopes to offer its programs to students from China, India, Mongolia, and the former Soviet republics, according to Sergei Verkhovets, head of international relations.

This semester, however, all 70 of Sukovataya's students are physically in her classroom. That means she spends 30 hours a week, including Saturdays, teaching in labs and lecture halls. "There is very little time for research," she says. "Still, I have not dropped my scientific research completely" on bioluminescence, which she carries out at the RAS institute. "After all, a teacher ideally should be up to speed on research and not just what is written in textbooks."

Echoing her student, she adds that the new software is not a substitute for a teacher. "Some teachers think, 'Now the software has appeared, I am not needed anymore.' But, in reality, it is the opposite. The teacher is still the central figure."

Helping hands. Irina Sukovataya has used money from teaching awards to develop new instructional materials.

–BRYON MACWILLIAMS

Bryon MacWilliams is a writer in Moscow.

CREDITS (TOP TO BOTTOM): MARCELO LEITE; BRYON MACWILLIAMS

Global reach. Campus signpost shows how far Leslie Lekala will go to teach physics.

SOUTH AFRICA

'I Wish … I Could Give [Them All] Computers'

Leslie Lekala teaches physics to thousands of students whom he'll never meet, using distance learning to help overcome the legacy of apartheid

PRETORIA, SOUTH AFRICA—As a boy, Leslie Lekala walked 2 hours each way to high school. Three decades later, he's still involved in distance education. But this time, he's teaching physics to thousands of students scattered across Africa.

Lekala's high school in remote Limpopo Province offered no physics or higher math, and his first lab course was at the University of the Witwatersrand (Wits), where he earned degrees in physics and mathematics. Now, he's a senior lecturer at the University of South Africa (UNISA), the continent's largest distance-learning institution and one of the world's megauniversities. But whereas some of those giant universities are switching to Internet learning, most of UNISA's assignments are still sent and received by snail mail. "I wish I had a magic wand and could give computers to each of our students," says Lekala about his first-year class of 1000 students. "But many of them simply don't have ready access to the Internet."

UNISA's enrollment of nearly 210,000 includes about 14,000 science or engineering majors. Many students can't afford, or don't qualify for, admission to other universities. Despite losing the cream of the crop to the nation's elite residential universities, Lekala says about 20% of his first-year students will major in physics, and some will go on for advanced degrees. That leaves Lekala, 46, with the enormous challenge of giving them a good foundation.

One obstacle is explaining difficult concepts without benefit of classrooms or face-to-face sessions. Lekala's most potent tool, for the relatively small percentage of students who seek help, is the telephone. "It's so much easier when you are in front of a chalkboard," says the soft-spoken Lekala. "By phone, you must be very clear in your explanations."

Although Lekala speaks seven of South Africa's 11 official languages, he wrestles to translate some physics concepts. Force and power, for example, are expressed by the same word in some African languages. "You have to explain the distinctions," he says. UNISA administrators are considering more "extra learner support" programs that would bring Lekala and his colleagues closer to students, including additional satellite classes, face-to-face tutoring, and online discussion groups. Many students would like more contact with their lecturers. "It is often difficult to outline your problem to a lecturer over the phone," says Ditlhase Frans Masita, who earned both his B.Sc. and master's degrees in physics at UNISA and plans to start work there soon on his Ph.D. Another problem, he says, is that "there are very limited resources" for lab work at UNISA, requiring upper-level students to travel to Johannesburg to use labs at Wits University. (Students travel to one of several UNISA testing centers around the country for final exams.)

Indeed, teaching lab sciences poses a major problem for many distance-learning universities. The acting dean of UNISA's science and engineering college, mathematician Ian Alderton, says students have access to software for learning basic lab procedures and "home experiment kits" for first-year physics. Many courses also require a 2-week intensive laboratory course, taken either at UNISA or another approved site.

For many first-year students, the lack of familiarity with lab work is a legacy of the apartheid regime, which invested little money for teaching science in mostly black schools. The weakness of disadvantaged schools continues: Last year, only about 15% of the 195,000 secondary school seniors in South Africa passed the qualifying exam in the physical sciences. This spring, the education minister—embarrassed that South African students ranked lowest on the last Trends in International Mathematics and Science Study exam, pulled out of the current round. Meanwhile, the ministry has established a nationwide network of 102 "Dinaledi schools"—similar to science-magnet high schools in the United States—to boost the study of math and physical science.

Poor preparation is not limited to students at distance-education universities. Geology professor Nicolas Beukes of the University of Johannesburg says some students are allowed to spread their one-term introductory geology course over a full year to have more time to master basic concepts. Similar "bridging" programs are being considered at UNISA.

Even if the quality of new science students can be improved, the number of students interested in science—especially the physical sciences—has been shrinking. Alarmed, the South African Agency for Science and Technology Advancement is working with both primary and secondary schools on programs to interest talented students in science. Gillian Arendse, a physics lecturer at Stellenbosch University who leads interactive sessions with high school students as well as workshops for teachers, says, "It's a challenge to attract the brightest students into science."

The goal of keeping talented students in school is one reason Lekala spends two-thirds of his time on teaching and administrative work. Even so, he manages to conduct research in his field of few-body physics, attend conferences, and stay in touch with colleagues around the world. He also reserves time for the 10 students typically in his higher-level classes. "These students know the basic concepts and tend to have better access to the Internet," says Lekala. "But it's still a challenge to teach them well."

–ROBERT KOENIG

CREDIT: R. KOENIG/SCIENCE

AUSTRIA

'Can't Have a Career . . . Without English'

Katrin Schäfer helps students acquire the skills they need to live and work in a global scientific community

VIENNA, AUSTRIA—"*Danke, sehr gut,*" says Katrin Schäfer, nodding to Christopher Schmied that he's up next. Schmied walks to the front of the class and takes a deep breath before launching into a description of his undergraduate research project, a social psychology experiment to test people's perception of facial hair. But the air seems to thicken like molasses as he switches to his Austrian-accented English.

The previous presentation had crackled along in German, with lightning-fast questions, answers, and even a few jokes zinging between Schäfer, a biological anthropologist, and her undergraduate students. But the easy fluency falls away as the class wrestles with a foreign language. In the polyglot discussion that follows, Schäfer switches between German and her crisply fluent English as needed to keep the conversation going.

Schäfer is a strong proponent of teaching science undergraduates here in English as a way to produce better prepared graduates and help the university attract the brightest students from across Europe. "You simply cannot have a career in the sciences without fluent English," says Schäfer, who grew up in Germany but moved to Vienna in 1988, "and the sooner you start, the better."

Walking down the hall after class, Schäfer daydreams about spending some time on her own research, the evolutionary forces that shaped the modern human skull. This is a "luxury" for which she carves out "10% of the week," an amount typical for someone past the grueling habilitation period but still early in her career. But students suddenly descend like hawks from every direction, desperately seeking final tweaks to posters that they must finish before accompanying her to an upcoming anthropology conference in Philadelphia. She's by their side until 7 p.m., looking over the English-language explanations of their work.

"I'm happy to do this because I want them to have a different experience from what we had," she says. As an undergraduate in this department nearly 20 years ago, says Schäfer, it was "sink or swim." Students were responsible for finding a faculty mentor and developing a research project that would lead to a Ph.D. "We were all so confused, and many of us dropped out or failed." Today's students have "wonderful advantages," she says, including guaranteed mentoring and exposure to international students and visiting scientists. "They also have more choices. Only about 10% go on to do a Ph.D., and that's fine. The [undergraduate] degree is still valuable."

Undergirding these new resources is a shifting landscape of language. Only a few generations ago, when cutting-edge research was published in journals with names like *Angewandtechemie*, proficiency in German was a must for serious science students around the world. Times have changed. Now most researchers acknowledge that English is science's lingua franca.

Auf Englisch, bitte. Katrin Schäfer (*left*) believes that teaching undergraduates in German puts them at a disadvantage.

But switching over to an English-based curriculum will not come easy, says fellow University of Vienna (UV) anthropologist Karl Grammer. "The opposition … will be very high," he says, "mainly because it would mean giving up German as a scientific language." Faculty members would also have to redo all their teaching material. Grammer favors a bachelor's program in German with an optional switch to English at the master's level.

Georg Winckler, an economist and rector of UV, would like the faculty to embrace what he calls "multilingualism": courses taught in German, with visiting professors lecturing in English. "The students can ask questions in German," he says, "and the professor must be able to understand but can answer in English." But at the moment, the decision is left to each department. Some, such as the molecular biology program, have already switched to English for their lectures.

Although Schäfer agrees that a melting-pot approach would help attract international students, she sees a deeper problem with holding on to the mother tongue. By requiring German language ability, candidates for new university positions are "limited to the German-speaking world." That factor helps explain why Austrian universities are filling with German scientists. "I have nothing against Germans; after all, I'm one of them," she says, "but if we're going to be a competitive research university, we need people from all around the world." The Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, has recruited a "world-class faculty," she says, by "doing everything in English."

This linguistic battle is taking place as UV and other universities on the continent struggle to restructure their degree programs. The current undergraduate system, identical to that of Germany to the northwest, is 5 years of study and a research-based thesis culminating in a degree called the Diplom. But in line with the "Bologna process"—an overarching plan to promote academic mobility in Europe by standardizing degrees that grew out of a 1999 gathering of education ministers in Bologna, Italy—the Diplom will be reduced to a 3-year U.K.–style bachelor's degree. To fill the gap between the bachelor's and the Ph.D., universities are introducing a buffet of 2-year master's degrees.

Although that transition may be a rocky one, everyone agrees that the status quo no longer works. It's a wrenching concession for an institution established in 1365 that helped spawn the current Anglo-American system. Now the tables are turned, Schäfer admits, "but it is for the best."

–JOHN BOHANNON

CREDIT: J. BOHANNON/SCIENCE

INDIA

Beyond Islands of Excellence

Nandula Raghuram's hands-on approach gives students a solid foundation for a biotech career

NEW DELHI, INDIA—Housed in the narrow by lanes of the old walled city of Delhi, where the modern Metro train and the horse-drawn cart compete for space, Nandula Raghuram's teaching laboratory provides a haven for three dozen budding biotechnologists. Hampered by inadequate supplies and equipment, the 42-year-old molecular biologist at Guru Gobind Singh Indraprastha University (GGSIPU) here makes do. Absent the large, expensive glass columns normally used for gel filtration, students separate proteins using ordinary surgical syringes whose ends have been plugged with latex tubes and tied off with thin rubber bands. "It's a low-cost, low-tech solution, but it allows each student to get his hands wet," he says.

Raghuram is an atypical teacher in a typical Indian university. Born into a middle-class family from a small town in southern India, Raghuram earned a Ph.D. in 1995 from Jawaharlal Nehru University in New Delhi and worked for industry before becoming a science policy analyst at the Center for Science and Environment here. He has also toiled as a science writer and is active in the Society for Scientific Values, a nongovernmental watchdog body that investigates scientific misconduct in India. In 2002, he became a Reader (the equivalent of an associate professor) at GGSIPU, created in 1999 as the youngest of six universities in this capital city. It allowed him to return to what he calls "my first love: doing and teaching science."

He devotes half of his 6-day, 65-hour workweek to teaching bachelor's and master's degree students at the university's School of Biotechnology, which accepts only 1% of the applicants for its 35 slots. That stiff competition reflects the popularity of the university's applied sciences curriculum and the success of graduates in finding good jobs, says Prakash Chand Sharma, dean of the school. In 2004, Raghuram was voted the university's best teacher based on what he believes is his success in answering a seemingly obvious question that few Indian professors actually ask: What do we want our students to learn in this class, and what skills do they need to acquire for that learning to take place?

Raghuram prefers using chalk and blackboard rather than PowerPoint presentations, explaining that his job is "not to show off my techie skills but

CHINA

'It's Important to Ask Students To Do Some Work on Their Own'

Yun Ying has pioneered a course that forces physics students to take the initiative and teaches them the English that they will need

NANJING, CHINA—China is in the midst of one of the most remarkable expansions of higher education ever attempted. And although Yun Ying, a semiretired professor of physics education at Southeast University in Nanjing, may be only a bit player, she's passionate about reforming science education. And she has a lifetime of experience.

In her nearly 6 decades as a teacher, she's weathered the Great Leap Forward and Cultural Revolution and benefited from China's opening to the West. Now, the 82-year-old Yun is leading her own minirevolution. Her introductory physics course addresses a national priority, namely, to foster economic growth by producing not just more, but more creative, scientists and engineers.

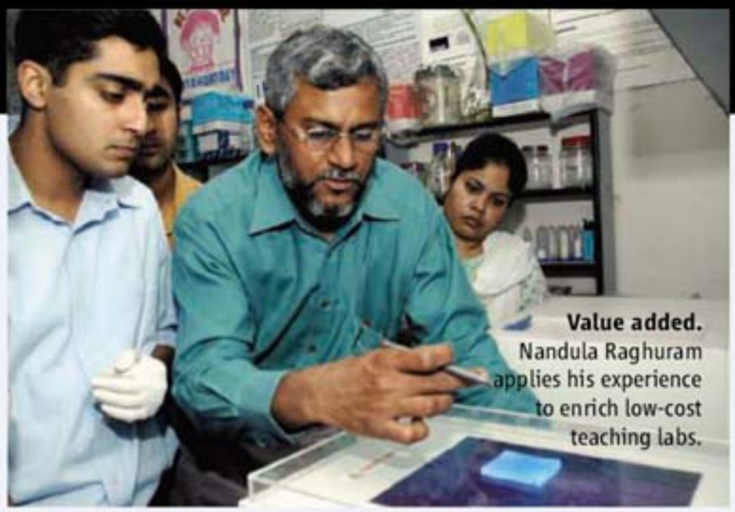
Yun has been wrestling with the challenge of revamping physics teaching since she returned from a 1980 tour of major U.S. research universities, which convinced her that Chinese students who hoped to study abroad needed to learn English tailored to those academic subjects. She also realized that "it is very important to ask the students to do some work on their own initiative."

Those two principles underlie her "Bilingual Physics With Multimedia" text and CD-ROM, a freshman course she has been developing since the mid-1980s that has been adopted by 10 Chinese universities. The course not only teaches the English that students need to discuss physics but also requires students to research physics topics and present their findings to the class. That's a dramatic change from the memorization demanded in typical introductory science courses. "There are no other texts like this for physics" in China, says engineer Xue Jingxuan of the Institute of High Energy Physics in Beijing, who is also concerned about science education in China. Xue says few university teachers put time and effort into developing course materials.

Creating a course may seem insignificant compared to the challenge of reinventing Chinese higher education. University enroll-

History lessons. Yun Ying has spent 60 years working to improve physics education.

CREDIT: COURTESY OF YUN YING



Value added. Nandula Raghuram applies his experience to enrich low-cost teaching labs.

CREDIT: P. BAGLA /SCIENCE

to explain concepts through discussion." His first Ph.D. student, Ravi Ramesh Pathak, calls him an "unconventional guide who gives his students total freedom for a holistic learning experience." But Raghuram laments "the declining popularity of teaching as a profession" among his peers and society as a whole.

In addition to teaching, Raghuram spends a quarter of his time doing basic science research on how plants take up and use nitrogen. With help from his three current graduate students, his lab has published more than 20 papers. The university pays his salary, and his research is funded by grants from various sources, including the Council of Scientific and Industrial Research and the Department of Science and Technology. The rest of his time is devoted to administrative duties, he says, including attending to students and colleagues who cluster around his small office sandwiched between the registrar's and vice chancellor's office.

"Raghuram is both a good communicator and researcher," says Sharma. But his many responsibilities, Sharma adds, means that "his teaching does suffer at times."

The School of Biotechnology offers students no formal career counseling, so Raghuram also tries to give them guidance. Sharma says about a third of the biotech graduates remain in school for a Master of Business Administration (MBA) degree, and a third go abroad for advanced degrees in the life sciences. The remainder go to work for companies or another institution. Ironically, Raghuram has never studied overseas, although he has traveled widely. "Doing science differently in India is what I have strived for while ensuring that my students also grow and blossom," he says.

GGSIPU is part of the government's plan to double over a decade the 9.2 million bachelor's degrees awarded each year. And Raghuram is motivated by the fact that GGSIPU students don't have the stellar background of those who vie for the 5000 or so coveted spots each year at the seven elite Indian Institutes of Technology across the country. He hopes that his work at GGSIPU will help the country build a system of higher education that shatters the current pattern of "islands of excellence in an ocean of mediocrity." Syringe by syringe, he's doing exactly that. **–PALLAVA BAGLA**

ments have jumped sevenfold since 1998, to 21 million in 2005, according to the Ministry of Education. Not surprisingly, classes are crowded, teaching loads are heavy, and building sprees have left many universities with staggering debt loads. And although funding has risen, it hasn't quite kept pace with the rising numbers, leading some universities to increase tuitions and try other means of raising funds.

But many officials say that the bigger challenge lies in reforming outdated curricula and teaching methods, particularly in science, technology, engineering, and math. Teaching methods and curricula still emphasize memorization, especially at the freshman and sophomore levels, and the goal is to foster creative researchers capable of making discoveries at both the basic and applied levels. "We have to have our own intellectual property," explains Rao Zihe, a structural biologist who is president of Nankai University in Tianjin. Rao fears that a dearth of homegrown creativity will forever relegate China to the status of refining innovations made elsewhere.

Yun is well equipped to take on that challenge. A 1947 physics graduate of Furen University, she spent 1 year in a master's program before joining what later became Southeast University. (Teachers with only a bachelor's degree were not uncommon at the time, although most university professors now hold Ph.D.s.) She had taken English since primary school and thought it appropriate for science courses. But after the Communist Revolution, she says, "we all learned Russian."

Yun's course deviates from the traditional approach in Chinese schools, notes Xue, in which "those who can memorize get better scores [on tests] than those who learn the text creatively." The textbook contains standard freshman-level lessons in momentum and energy, harmonic motion, and wave-particle duality. All explanations are given in depth in English with Chinese translations of key passages. The CD-ROM includes video clips illustrating various principles.

The videos "gave a deeper understanding of how the laws of physics apply to daily life," says Hu Te, a Southeast software engineering student who took Yun's course. Even more unusual is the requirement that students select a topic, research it on their own or in a small group, and then present their findings in a class seminar—all in English. Other students can ask questions, make comments, or challenge the conclusions—unprecedented conduct for Chinese undergraduates, says Xue.

Despite the use of English, Yun hasn't watered down the content. Some of that may be due to Southeast's ranking as one of the country's top 10 comprehensive universities, with a particular strength in engineering. Li Xin, a sophomore honors student who was required to take Yun's course as a freshman, says it was completely different from his high-school physics courses, which were "just theories and equations and formulas—and boring."

Du Yuan, another honors student required to take the course, says the opportunity to independently research a topic—his was the "Yesterday, Today, and Tomorrow of Space Flight"—was a rare treat for a freshman. And Hu says the vocabulary learned in Bilingual Physics allows him "to read about the theories of Nobel Prize winners, which haven't been translated into Chinese."

Yun is pleased with the positive reaction to her course. Two years ago, she offered a teacher-training course for schools considering adoption of the text and CD-ROM, and now she's working on a teaching and learning guidebook.

Xue speculates that the course hasn't attracted more attention because old habits die hard among university professors, who he says are focused on their research. But the increasing number of faculty members who were trained in the United States or Europe has sparked interest in reforming teaching at Chinese universities. A one-semester course taken primarily by engineering students may have a limited impact on Chinese education, he admits. But for those calling for an educational revolution in China, it's a good place to start.

–DENNIS NORMILE

SOUTH KOREA

'A Strong Voice' For Course Reform

Lee Duckhwan fights to keep science in the forefront of what students are asked to learn in high school and college

SEOUL, SOUTH KOREA—A professor at a prestigious engineering college is scribbling on a chalkboard, so the story goes, when a puzzled freshman looks at the integration sign in one of the equations and asks, "What is that curly symbol?"

Lee Duckhwan, a chemist at Sogang University here, says the story, which he swears is true, illustrates the large number of South Korean students who arrive ill-prepared for college-level mathematics. He blames the "unprecedented crisis" on a 1997 national curriculum that lifted requirements on many high school science and math courses. With two-thirds of students now avoiding science altogether in 11th and 12th grades, Lee says university science departments have been forced to offer remedial courses before students can tackle the regular curriculum.

The state curriculum leaves little to the imagination, says Lee, who was asked to write a high school chemistry textbook. "They dictated what to put in the textbook, down to the smallest detail," says Lee, whose complaints went unheeded. Last year, when the education ministry conducted a review of the curriculum, Lee penned commentaries and rallied colleagues in hopes of strengthening the science component. He also served on a science panel—one of 1300 experts on a dizzying 100 review groups. "Duckhwan helped us find a strong voice," says physicist Oh Sejung, dean of natural sciences at Seoul National University (SNU).

But in February, when the ministry unveiled new standards to take effect in 2009, the requirements had been softened further. For instance, 11th- and 12th-year students will be able to opt for a course in home economics or business management instead of science or math. The review "was bogus," Lee fumes.

The criticism is unwarranted, says an education ministry official, who notes that the new standards will add 1 hour a week of 10th-grade science. And she asserts that most 11th- and 12th-year students take math and science. Lee replies that the extra hour was added to "repair" what had been dropped from the 1997 curriculum and that the ministry's statistics "do not correctly reflect" science enrollments.

Lee is no Johnny-come-lately to science literacy. He earned a Ph.D. in theoretical chemistry at Cornell University in 1983 and spent 2 years at Princeton before landing at Sogang. Not content to limit himself to his field of nonlinear optics, Lee has translated several popular science books into Korean. Three years ago, he founded the country's first graduate program in science communication, and last year, he led preparations for the International Chemistry Olympiad in Seoul.

Leading the charge. Lee Duckhwan has rallied colleagues in an effort to bolster science in Korea's national curriculum.

Few countries outdo South Korea in these Olympiads or on standardized science and math tests, and until a decade ago, "every student had to learn every branch of science," says Kim Jin Seung, a physics professor at Chonbuk National University in Jeonju. But science got caught up in a broader school reform movement sweeping across South Korea and Japan in the mid-1990s that sought to do away with heavy memorization, Saturday classes, and unbending course requirements. The outcome, says Lee, enshrines free choice as a tonic for low creativity—creating a new problem without solving the old one.

Not only are high school students opting to take less science, but there are also fewer students to go around. The number now taking the college entrance exam is nearly half the level in the 1980s, when a postwar baby boom filled campuses. And the ratio of science to liberal arts majors has flipped from 6:4 to 3:7 in the past decade, says Kim Dohan, president of the Korean Mathematical Society and a professor at SNU.

Two science universities—the Korea Advanced Institute of Science and Technology and Pohang University of Science and Technology (POSTECH)—are buffered to a degree by drawing heavily from the country's 19 elite science high schools, and SNU also gets more than its share of these science-savvy teens. Even so, Oh says, one in five SNU science and engineering freshmen needs remedial algebra before taking college-level courses.

Lee and others worry that the revised curriculum will further dilute science teaching for most students, and the heads of six science organizations have demanded revisions in the new curriculum. "By making these [science and math] courses optional, it's a bad signal to students that math and science are not important," says mathematician Min Kyung Chan of Yonsei University in Seoul. Ironically, these changes coincide with a doubling of government spending on research over the last 5 years.

The status quo, Lee predicts, will lead to a steady erosion of science literacy in South Korea. That could lower the level of public discourse on many important issues—from nuclear waste disposal to the routing of high-speed train lines—with a scientific component. Lee also fears that the growth in South Korea's research capacity will be stifled.

In the meantime, universities are scrambling to drum up interest in science. POSTECH launched an undergrad research program that awards $4000 to each of 30 students, who may choose their own topic and adviser from anywhere in the world. "The students can spend the money however they want. They decide everything," says POSTECH's dean of student affairs, Kang In Seok, who designed the program. But such efforts may not be enough to offset rising levels of apathy toward science among the young.

–RICHARD STONE

CREDIT: COURTESY OF LEE DUCKHWAN

DNA for all. Toyoko Akiyama champions laboratory courses for nonscience majors.

CREDIT: D. NORMILE/SCIENCE

JAPAN

Spreading Knowledge of Science and Technology

Toyoko Akiyama fosters a movement to counter specialization by giving all students a real taste of her biology lab

YOKOHAMA, JAPAN—Toyoko Akiyama began her recent biology class at Keio University here by explaining DNA analysis, including how to amplify a DNA fragment using the polymerase chain reaction (PCR). Then she demonstrated how to use a micropipette. Next stop was the lab, where all 58 students collected cells from their own cheeks and prepared samples for the PCR thermal cycler. When the students met again, they would learn whether they carried a particular polymorphism within a certain region of their own genome.

Akiyama's class would not be unusual for budding biologists. But her students are majoring in economics, literature, marketing, and political and social sciences; some have taken no science courses since junior high school. "We think all educated adults should have some understanding of science and scientific procedures," says Akiyama, explaining an educational philosophy that goes back to the school's founding in 1858 by Yukichi Fukuzawa, a leading intellectual and entrepreneur who recognized the importance of the natural sciences to Japan's modernization.

In an era of increasing academic specialization, Keio is a rare exception among Japan's private universities in offering lab courses for nonscience majors. And most of the country's roughly 100 national universities virtually eliminated science course offerings for nonscience majors in a wave of restructuring a decade ago that closed departments of general studies, explains Toshio Hyodo, a University of Tokyo physicist long concerned about science education. But "universities are reconsidering those reformations," he says. As both students and educators rethink the value of a well-rounded education, they could once again embrace the type of courses that Keio has been offering for 50 years.

Akiyama, a biophysicist, is not an obvious choice to be leading the charge. Shoulder-high even to most of her female students, with a ready smile and quiet demeanor, she and her colleagues here on Keio's Hiyoshi campus technically belong to the faculty of law and not the science faculty, located at another of its five campuses throughout the Tokyo area. (Considered one of Japan's top private universities, Keio counts many prominent business people and politicians among its alumni.) She admits to an occasional longing to "talk to students who are science majors," which she indulges by giving seminars and courses in her specialty of pigment cells and their developmental processes. And she tries to carve out a few days a week to pursue her research, although she concedes that "lots of meetings" interrupt that schedule.

But teaching biology to nonscience majors is her passion, one that she's pursued for 30 years. She puts considerable time into developing lesson plans. Her curriculum is more topical than systematic, and experimental themes resonate with current issues. In setting up the DNA experiment, she mentioned how DNA analysis determined that recently recovered human remains were probably not those of a famous kidnapping victim.

"DNA is in the news, and this helps us understand what it is all about," says Taro Yamanaka, a 19-year-old political science major who was handling a pipette for the first time. Akiyama says she enjoys watching students discover a new interest and nurturing an antidote to a growing alienation from the natural sciences among Japan's youth.

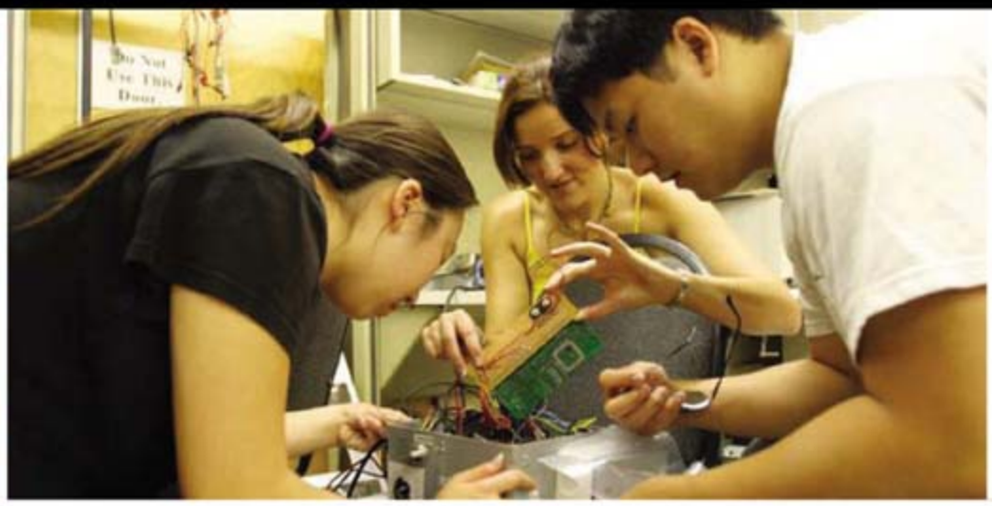
One of the challenges Akiyama faces is a wide range in the amount of high school preparation her students have had. Many students devote all their time to the subjects they need to master to pass specialized university entrance exams. So when they enter Akiyama's classroom, some students have only junior high science courses to their credit whereas others have 3 years of high school biology. "Some students find the material too basic, whereas others struggle," she says.

On this day, everyone seems to be keeping up with the DNA analysis. Yamanaka says the lab work is a "refreshing change from studying law all the time." Classmate Kanako Arai, an economics major, hopes to meld her interests by studying the economic aspects of environmental issues. A 2005 survey done by the university of alumni 5 and 10 years after graduation found that 80% thought the natural science education was worthwhile, and 90% felt that the lab experiments were meaningful.

And the courses could get even better. In 2005, Japan's Ministry of Education awarded the Keio group a 4-year, $900,000 grant—supplemented by the university—to develop new experiments and to survey natural science education for nonscience majors both in Japan and overseas. "One of the objectives of the grant is to serve as a possible model for other universities," says Minoru Omote, a Keio physicist who is leading the project.

A model might not be sufficient, however. Student labs require benches, plumbing, and safety devices not needed in lecture halls. And the DNA experiment, for example, relies on centrifuges, hot-water baths, PCR cyclers, and pipettes, as well as consumables such as pipette tips and reagents. Akiyama also depends on three teaching assistants to help supervise the students' lab efforts. Lab classes "take a lot of money and space," says Akiyama. Given scarce resources, it may be hard to spread Keio's example.

–DENNIS NORMILE



NEWS

Straight Talk About STEM Education

Getting your hands dirty is one of many keys to a successful undergraduate education in the sciences, says this panel of U.S. educators

SCIENTISTS FROM A VENERABLE WOMEN'S COLLEGE, AN ELITE liberal arts' school with a focus on science and engineering, and an expanding urban university might seem to inhabit different worlds. But when *Science* invited environmental scientist Stephanie Pfirman of Barnard College in New York City, mathematician Daniel Goroff of Harvey Mudd College in Claremont, California, and biochemist Michael Summers of the University of Maryland, Baltimore County (UMBC), to participate in a roundtable discussion, the three distinguished educators had no trouble finding common ground in describing their efforts to improve undergraduate science, technology, engineering, and math (STEM) education. Here is an excerpt of that conversation. The full text is available online at www.sciencemag.org/sciext/undergrad_education07. **–JEFFREY MERVIS**

What are the challenges facing institutions trying to increase participation in STEM fields?

Summers: We have 12,000 students at UMBC, and there can be 300 or 400 students in a session of freshman chemistry. But when students come to college with an interest in science and take these big courses, some of them get turned off very quickly. They haven't figured out what it takes to excel at the college level, and it can be disheartening if you come from a population that isn't well represented in the sciences and you look around and see other students performing well.

What's the freshman experience like at Harvey Mudd?

Goroff: All of our students come here expecting to do something in a STEM field, and our core program aims to prepare them to address the big problems in the world. In addition, anything we can do to erase the myth of a lone genius as the exemplar of what it takes to succeed in science and show them that science is done in connected communities rather than by someone holed away in an office really helps with the retention problem.

Pfirman: For many liberal arts students, their only exposure is through a science requirement. Unfortunately, these classes have often dumbed down the science. At Barnard, there are first-year seminars that had traditionally been offered by the English and humanities departments, and we decided to offer one in the sciences that sneaks in the science along with the rest of the subject matter. After the class, the students reported they felt a greater connection to science and saw a greater role for science in their lives.

How hard is it to revise an intro course, and what are the limiting factors?

Summers: Part of it is knowing what to do. NSF [National Science Foundation] and HHMI [Howard Hughes Medical Institute] have funded pilot projects that are, in effect, experiments designed to modify the curriculum, for example, to make biology more quantitative. What's needed now are the data that show the impact of these programs.

Pfirman: Faculty really want to be effective. It's not just what content is appropriate but also how to deliver it and how to assess it, so that you can do a better job next time. … What would really help is a way to find out what's out there that has worked well. I was just looking for a lab module to go with a unit on the ocean's role in the carbon cycle. It's a fairly basic concept. But I couldn't find anything on the Web.

What would you recommend for a faculty member who wants to do something but doesn't know where to look?

Summers: When we decided that we wanted to try to retain more students in chemistry, we looked at bridge programs—a summer program for incoming freshmen—including how to study in groups. Hal White at the University of Delaware has been doing good things with small groups. But these things cost money, and you need teaching assistants. So we had to go to the administration to get money to test this experiment. But now that we've done it and put the results on the Web, I would hope that other faculty could take the data and show it to the dean and say, "This is what worked there, and I think we can do the same thing here."

Goroff: I'd encourage faculty to get involved with national groups, too. It doesn't make sense to reinvent the wheel, much less the flat tire. Project Kaleidoscope [pkal.org] is a network of young faculty who are sharing ideas. Project Next [maa.org] is another such effort.

CREDITS (LEFT TO RIGHT): KEVIN MAPP/HARVEY MUDD COLLEGE

Flying high. (*Opposite page*) Harvey Mudd students test carbon fiber poles and the output of LED lights in a project for the Federal Aviation Administration. (*Above, left*) Meyerhoff graduate fellows take a break from the lab for an outing to Harpers Ferry in West Virginia. (*Above, right*) Barnard's Maggie Chan collects and characterizes sediment samples from the Hudson River bed near Manhattan.

UMBC is one of several institutions that are trying to increase retention rates among minority students and narrow the achievement gap. How is that working?

Summers: Our president, Freeman Hrabowski, began with the premise that there are a large number of high-achieving minority students, in particular African Americans, who are just not being retained once they enter college to study science. He goes after them in high school. He brings them to campus with their families, and we start talking about earning a Ph.D. degree.

Over the past 20 years, we've graduated more than 500 students, a large percentage of whom have gone on to get graduate degrees. … And we've tried to quantify the experiment with controls. We bring about 200 students to campus and make about 100 offers—and these are full scholarships—knowing that only about 50 will actually join the program. And before we make an offer, we make parents sign a waiver that says even if you turn us down, we want to be able to track them. So now we have a database. It turns out that if they go elsewhere—and these kids get offers from Ivy League schools and everywhere else—they are half as likely to graduate with a science or math degree and more than five times less likely to go to graduate school.

Have you identified elements that make a difference?

Summers: We haven't identified what happens to them on other campuses. But at UMBC, the top thing is making sure that they are academically successful in the freshman courses. It starts with a summer bridge, and it includes getting students involved right away with research projects.

A lot of them have taken AP classes and could place out of chemistry. But they take the freshman courses, anyway. And the key is doing well in those first courses. Because if they are successful, then the research faculty are eager to get them involved in their labs.

What if they're not successful?

Summers: There are counselors in their first semester who keep track of every pop quiz and test that students take. If they have problems, they are immediately paired up with other classmates who are doing well in a particular subject. And we pay seniors to be mentors. We'll also call the parents, and there's a parents' association that provides money and time. This is a group effort.

People have accused the program of cherry picking by only taking the most able students. What's your response?

Summers: We are taking students who are most likely to succeed based on high school performance. But we are only taking a small fraction of that pool—we get 2000 nominations a year and about 800 applications [for approximately 50 slots]. So in a way, we are cherry-picking. But we don't apologize for it. And we only take a small percentage. People should be cherry-picking in every state.

"I see the environment as the liberal science field of this century. It brings together all the disciplines … and gives students a link to their own lives."

—Stephanie Pfirman, Barnard College

Are environmental programs a good way to attract students into STEM fields?

Pfirman: I see the environment as the liberal science field of this century. It brings together all the disciplines, and you have a problem-solving focus that makes them naturally work together. It also gives students a link to their own lives.

How career-oriented are your students, and how much should they be?

Pfirman: We try to give them the skills that will allow them to be successful in anything they do, whether they're balancing a checkbook or training to be an environmental economist.

Goroff: Nature doesn't give us the great problems of the world labeled as physics or chemistry. So we want students to have that broad background. But having an in-depth experience is useful, too.

CREDITS (TOP TO BOTTOM): MICHAEL F. SUMMERS; M. STUTE; BRUCE GILBERT

Even if students aren't thinking about careers, however, we need to be paying more attention to them, because the opportunity costs to go into a science career are much different than for someone in China or India. We shouldn't be surprised if students are thinking about law school or business school if we haven't paid attention to finding ways of making a science career fulfilling and rewarding—and I'm not just talking about money—without first having to spend 10 years as a graduate student and a postdoc.

How career-oriented are UMBC students, and does that push them into or out of STEM fields?

Summers: We have some students who have known since kindergarten that they want to be a medical doctor, and that's fine. But in general, I think we need to be careful about pushing students into careers at too early an age. I know from talking to some of my graduate students from other countries that they are tested and channeled at a very early age into either science or nonscience tracks. I think it's good for students to have the opportunity to get excited by a course or a professor and have a chance to go into science.

How well are institutions doing in tracking student achievement and what happens to them after graduation?

Summers: I don't know if there's any real tracking of our general population once they leave. We certainly track those in our Meyerhoff Scholars Program. We know where they are in grad school and their postdocs and when they apply for faculty jobs, because we want to know if we are being successful.

I don't think we take the same scientific approach to our teaching responsibilities as we do to our research. In research, we are evaluated based on our performance. If we write an NSF or NIH [National Institutes of Health] grant, we have certain objectives, and our peers look at those metrics when they judge our proposals, and later on they ask if we have been successful. Well, a major part of our job is to teach and educate. I don't think it should be mandated from the top. But I think we should approach teaching as we approach our science and think about what's important. If we're not retaining students in our own areas, then we're not doing our jobs.

"If we're not retaining the students that we're training, then we're in trouble."

—Michael Summers, University of Maryland, Baltimore County

What does accountability mean at Barnard?

Pfirman: One thing we do is track our majors, and we invite them back to talk about their careers. But we haven't asked them to reflect on what was it that made them choose a particular career path. We do an exit survey when they leave Barnard, but I think that their perspective a couple of years out would be very valuable.

We're also trying to understand better what students are learning in their classes. We're working with the Consortium on High Achievement and Success to understand why, for example, minority students might come in with high SATs but then tend to underperform, especially in large introductory courses. One interesting approach is to ask students to come up with a flow chart or a timeline to depict what they are studying, or to reflect on what they have learned. We're doing it in an environmental measurements class, in which students take samples from the Hudson River and analyze them, and it helps me to better evaluate their work.

What can universities do to make teaching a more attractive option for their STEM students?

Goroff: I run into STEM students all the time at the best institutions who are passionate about their subjects and who would love to make a career out of teaching. But they have trouble figuring out what a career like that would look like. So I think we need to take some responsibility and think about how these labor markets work and in particular the type of communities that exist to support that goal. In many countries, being a teacher is a very honorable and community-based activity, and teachers get together all the time to improve their craft. But that happens very little in the United States, and that's too bad. There are some programs, like Math for America or Teach for America, where you can see that progression and see how to make it a career.

Is teaching seen as a successful outcome of the Meyerhoff program, or is it more oriented toward research careers?

Summers: I think that there is some tension there. We're trying to get our students to feel passionate about science, which involves doing experiments. We brag about our students who go to graduate school at Harvard. So if a student goes into the lab and gets excited about doing research, will they have that opportunity if they decide to go into teaching? Probably not. They will probably have huge teaching loads, and it's not a glamorous or well-paid profession.

The clinic program at Harvey Mudd is a good example of providing research opportunities for undergraduates. How does it work?

Goroff: It's a signature program that has been going for more than 40 years, and it allows students to partner with industry or a federal lab or venture capitalists—people who have real problems that they need to find an answer to. The importance of having a client like that really makes a big difference. … We've even turned the program into a global activity, using videoconferencing and e-mail. It not only gets them ready to work in ways that employers and graduate schools appreciate, it also allows students who may not have the highest grade point average to excel as part of a team.

How do you make sure that they don't become just another pair of hands?

Goroff: We give away the intellectual property rights, and we're not trying to make money on this. We spend a lot of time working with sponsors to pick problems that will work well for undergraduates, and we explain that sometimes finding out the original idea didn't work can be a tremendously valuable experience—and a tremendous help to the company, too.

There is continuing pressure on universities to eliminate programs aimed at special populations on the grounds that they discriminate against the majority population. Is that having an impact on the programs that you run?

Summers: At UMBC, we have had to worry about that a little bit. When the Meyerhoff program began, it was specifically for

CREDIT: TIM FORD/UMBC

black males, and then women, and then minorities in general. After the Banneker ruling [in 1994] shut down minority science programs at the University of Maryland, we decided to open up the program to all students who have an interest in diversity issues in the sciences. … If a white student qualifies, they have to decide if they want to do all the outreach and activities that are part of the Meyerhoff program—such as serving as tutors to inner-city minority students in the K–12 system and other monthly activities—or if they would rather take a general merit scholarship from the university. The program is now about 12% Caucasian and 12% to 15% Asian.

What about at Barnard?

Pfirman: We are a women's college, so opening it up to all our students just means more women. But I'm also working on a project to advance women faculty, and one thing we've discovered is that programs to help women are also helpful for men. So we've opened up the programs to both men and women, although we tend to get more women.

How do you extend a program that's existed for decades at one institution to the hundreds or even thousands of schools that might benefit from it?

Summers: We're trying to do that with a program funded by NIH and HHMI to increase retention rates among underrepresented minorities. We've had three meetings, hosted and attended by institutions that we felt were primed for changed. To participate, each institution first had to do a quantitative assessment of how women and minorities are doing on their campuses. The information wasn't easy to get, and many said they were surprised at how poorly their institutions were doing with respect to the achievement of minorities and women once they saw the numbers. In January, there will be another meeting for the institutions to present the outcomes of things that they have put in place. …

We call this "taking the show on the road." If you go to a typical scientific meeting, there might be 15,000 at the meeting. But only about 30 would attend a session on diversity. And they are usually the same people. After spending so much money for so many years trying to increase the number of minority scientists and seeing the number increase from maybe 1% to 2% in the sciences, federal agencies know they have to do things differently. … They have been able to build campfires at some institutions, and the question now is how do you fan the flames.

Pfirman: One critical need is to organize the resources and make them accessible. Many of us realize that there are ways to improve teaching, but it's hard to find that information. I know how to find out what's happening with Arctic sea ice, for example, but I don't know how to find the resources I'm looking for in the educational arena. We also need to engage the professional societies. They have these big annual meetings, but it's hard to attract more than the usual suspects to sessions on diversity or education. If there was some way they could elevate the issue, that would be great.

Why isn't there sufficient national leadership now, and what can people do to make it happen?

Goroff: I think it's always fashionable in Washington to concentrate on the dollar signs and to think that if we just spend more money, the leaders will appear and the programs will appear. But if you look at what happened to NIH after the doubling of its budget—which was a wonderful thing that is absolutely worthy of our support—what you see is a great deal of this winner-take-all kind of funding schemes and the same people getting more grants. I'd rather see us sending money to people who want to implement some of these good ideas at their institution.

Summers: When we interview people for a position, some postdocs tell us that their adviser has warned them not to get too involved with undergraduates because they will end up spending too much time on teaching, and that could ruin their career. Faculty at most research institutions are evaluated on the basis of papers published and presentations at meetings and service on review panels. There's a teaching component, but it's not weighted the same. And there's no component at all that addresses mentoring students in your lab.

What one or two things would you like to see happen in the next 5 to 10 years to improve undergraduate STEM education, and how would you measure it?

Pfirman: I think it's critical within each of our institutions that we have academic leaders on campus who understand the value of science and math and what resources can make a difference. If more science faculty are willing to step up to those leadership positions, that could make a big difference. We also need to think of our institutions as learning communities and get everybody involved in the learning process.

> "We [need] to erase the myth of a lone genius … and show [students] that science is done in connected communities."
>
> —Daniel Goroff, Harvey Mudd College

Goroff: We've mentioned community many times. And I think that being part of something bigger than oneself is very appealing. If we can dispel the myth of the lone genius working on their own and emphasize the social capital, that would be a step in the right direction. A second contribution would be more data about learning and mentoring.

Summers: In science, sometimes making the big discovery means being in the right place in the right time. But when it comes to our own disciplines and our students, we're all in the right place at the right time to do something. When a minority student makes a C, rather than thinking, "That's good, they've done all right," we could bring them in and ask them, "Why didn't you make a B? How can you do better?" If we're not retaining the students that we're training, then we're in trouble.

CREDIT: KEVIN MAPP/HARVEY MUDD COLLEGE

Are Women Really More Talkative Than Men?

Matthias R. Mehl,[1]* Simine Vazire,[2] Nairán Ramírez-Esparza,[3] Richard B. Slatcher,[3] James W. Pennebaker[3]

Sex differences in conversational behavior have long been a topic of public and scientific interest (*1*, *2*). The stereotype of female talkativeness is deeply engrained in Western folklore and often considered a scientific fact. In the first printing of her book, neuropsychiatrist Brizendine reported, "A woman uses about 20,000 words per day while a man uses about 7,000" (*3*). These numbers have since circulated throughout television, radio, and print media (e.g., CBS, CNN, National Public Radio, *Newsweek*, the *New York Times*, and the *Washington Post*). Indeed, the 20,000-versus-7000 word estimates appear to have achieved the status of a cultural myth in that comparable differences have been cited in the media for the past 15 years (*4*).

In reality, no study has systematically recorded the natural conversations of large groups of people for extended periods of time. Consequently, there have not been the necessary data for reliably estimating differences in daily word usage among women and men (*5*). Extrapolating from a reanalysis of tape-recorded daily conversations from 153 participants from the British National Corpus (*6*), Liberman recently estimated that women speak 8805 words and men 6073 words per day. However, he acknowledged that these estimates may be problematic because no information was available regarding when participants decided to turn off their manual tape recorders (*4*).

Over the past 8 years, we have developed a method for recording natural language using the electronically activated recorder (EAR) (*7*). The EAR is a digital voice recorder that unobtrusively tracks people's real-world moment-to-moment interactions. It operates by periodically recording snippets of ambient sounds, including conversations, while participants go about their daily lives. Because of the covert digital recording, it is impossible for participants to control or even to sense when the EAR is on or off. For the purpose of this study, the EAR can be used to track naturally spoken words and to estimate how many words women and men use over the course of a day.

In the default paradigm, participants wear the EAR for several days during their waking hours. The device is programmed to record for 30 s every 12.5 min. All captured words spoken by the participant are transcribed. The number of spoken words per day can then be estimated by extrapolating from a simple word count, the number of sampled sound files, and the recording time per sound file.

We addressed the question about sex differences in daily word use with data from six samples based on 396 participants (210 women and 186 men) that were conducted between 1998 and 2004. Five of the samples were composed of university students in the United States, and the sixth, university students in Mexico. Table 1 provides background information on the samples along with estimates for the number of words that female and male participants spoke per day (*8*).

The data suggest that women spoke on average 16,215 (SD = 7301) words and men 15,669 (SD = 8633) words over an assumed period of, on average, 17 waking hours. Expressed in a common effect-size metric (Cohen's d = 0.07), this sex difference in daily word use (546 words) is equal to only 7% of the standardized variability among women and men. Further, the difference does not meet conventional thresholds for statistical significance (P = 0.248, one-sided test). Thus, the data fail to reveal a reliable sex difference in daily word use. Women and men both use on average about 16,000 words per day, with very large individual differences around this mean.

A potential limitation of our analysis is that all participants were university students. The resulting homogeneity in the samples with regard to sociodemographic characteristics may have affected our estimates of daily word usage. However, none of the samples provided support for the idea that women have substantially larger lexical budgets than men. Further, to the extent that sex differences in daily word use are assumed to be biologically based, evolved adaptations (*3*), they should be detectable among university students as much as in more diverse samples. We therefore conclude, on the basis of available empirical evidence, that the widespread and highly publicized stereotype about female talkativeness is unfounded.

Table 1. Estimated number of words spoken per day for female and male study participants across six samples. N = 396. Year refers to the year when the data collection started; duration refers to the approximate number of days participants wore the EAR; the weighted average weighs the respective sample group mean by the sample size of the group.

Sample	Year	Location	Duration	Age range (years)	Sample size (N)		Estimated average number (SD) of words spoken per day	
					Women	Men	Women	Men
1	2004	USA	7 days	18–29	56	56	18,443 (7460)	16,576 (7871)
2	2003	USA	4 days	17–23	42	37	14,297 (6441)	14,060 (9065)
3	2003	Mexico	4 days	17–25	31	20	14,704 (6215)	15,022 (7864)
4	2001	USA	2 days	17–22	47	49	16,177 (7520)	16,569 (9108)
5	2001	USA	10 days	18–26	7	4	15,761 (8985)	24,051 (10,211)
6	1998	USA	4 days	17–23	27	20	16,496 (7914)	12,867 (8343)
					Weighted average		16,215 (7301)	15,669 (8633)

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Supporting Online Material

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Materials and Methods
Fig. S1
Table S1
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Wireless Power Transfer via Strongly Coupled Magnetic Resonances

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Using self-resonant coils in a strongly coupled regime, we experimentally demonstrated efficient nonradiative power transfer over distances up to 8 times the radius of the coils. We were able to transfer 60 watts with ~40% efficiency over distances in excess of 2 meters. We present a quantitative model describing the power transfer, which matches the experimental results to within 5%. We discuss the practical applicability of this system and suggest directions for further study.

In the early 20th century, before the electrical-wire grid, Nikola Tesla (*1*) devoted much effort toward schemes to transport power wirelessly. However, typical embodiments (e.g., Tesla coils) involved undesirably large electric fields. The past decade has witnessed a surge in the use of autonomous electronic devices (laptops, cell phones, robots, PDAs, etc.). As a consequence, interest in wireless power has reemerged (*2*–*4*). Radiative transfer (*5*), although perfectly suitable for transferring information, poses a number of difficulties for power transfer applications: The efficiency of power transfer is very low if the radiation is omnidirectional, and unidirectional radiation requires an uninterrupted line of sight and sophisticated tracking mechanisms. A recent theoretical paper (*6*) presented a detailed analysis of the feasibility of using resonant objects coupled through the tails of their nonradiative fields for midrange energy transfer (*7*). Intuitively, two resonant objects of the same resonant frequency tend to exchange energy efficiently, while dissipating relatively little energy in extraneous off-resonant objects. In systems of coupled resonances (e.g., acoustic, electromagnetic, magnetic, nuclear), there is often a general "strongly coupled" regime of operation (*8*). If one can operate in that regime in a given system, the energy transfer is expected to be very efficient. Midrange power transfer implemented in this way can be nearly omnidirectional and efficient, irrespective of the geometry of the surrounding space, with low interference and losses into environmental objects (*6*).

The above considerations apply irrespective of the physical nature of the resonances. Here, we focus on one particular physical embodiment: magnetic resonances (*9*). Magnetic resonances are particularly suitable for everyday applications because most of the common materials do not interact with magnetic fields, so interactions with environmental objects are suppressed even further. We were able to identify the strongly coupled regime in the system of two coupled magnetic resonances by exploring nonradiative (near-field) magnetic resonant induction at megahertz frequencies. At first glance, such power transfer is reminiscent of the usual magnetic induction (*10*); however, note that the usual nonresonant induction is very inefficient for midrange applications.

Overview of the formalism. Efficient midrange power transfer occurs in particular regions of the parameter space describing resonant objects strongly coupled to one another. Using coupled-mode theory to describe this physical system (*11*), we obtain the following set of linear equations:

$$\dot{a}_m(t) = (i\omega_m - \Gamma_m)a_m(t) + \sum_{n \neq m} i\kappa_{mn}a_n(t) + F_m(t) \qquad (1)$$

where the indices denote the different resonant objects. The variables $a_m(t)$ are defined so that the energy contained in object m is $|a_m(t)|^2$, ω_m is the resonant angular frequency of that isolated object, and Γ_m is its intrinsic decay rate (e.g., due to absorption and radiated losses). In this framework, an uncoupled and undriven oscillator with parameters ω_0 and Γ_0 would evolve in time as $\exp(i\omega_0 t - \Gamma_0 t)$. The $\kappa_{mn} = \kappa_{nm}$ are coupling coefficients between the resonant objects indicated by the subscripts, and $F_m(t)$ are driving terms.

We limit the treatment to the case of two objects, denoted by source and device, such that the source (identified by the subscript S) is driven externally at a constant frequency, and the two objects have a coupling coefficient κ. Work is extracted from the device (subscript D) by means of a load (subscript W) that acts as a circuit resistance connected to the device, and has the effect of contributing an additional term Γ_W to the unloaded device object's decay rate Γ_D. The overall decay rate at the device is therefore $\Gamma'_D = \Gamma_D + \Gamma_W$. The work extracted is determined by the power dissipated in the load, that is, $2\Gamma_W|a_D(t)|^2$. Maximizing the efficiency η of the transfer with respect to the loading Γ_W, given Eq. 1, is equivalent to solving an impedance-matching problem. One finds that the scheme works best when the source and the device are resonant, in which case the efficiency is

$$\eta = \frac{\Gamma_W|a_D|^2}{\Gamma_S|a_S|^2 + (\Gamma_D + \Gamma_W)|a_D|^2} = \frac{\frac{\Gamma_W}{\Gamma_D}\frac{\kappa^2}{\Gamma_S\Gamma_D}}{\left[\left(1 + \frac{\Gamma_W}{\Gamma_D}\right)\frac{\kappa^2}{\Gamma_S\Gamma_D}\right] + \left[\left(1 + \frac{\Gamma_W}{\Gamma_D}\right)^2\right]} \qquad (2)$$

The efficiency is maximized when $\Gamma_W/\Gamma_D = [1 + (\kappa^2/\Gamma_S\Gamma_D)]^{1/2}$. It is easy to show that the key to efficient energy transfer is to have $\kappa^2/\Gamma_S\Gamma_D > 1$. This is commonly referred to as the strong coupling regime. Resonance plays an essential role in this power transfer mechanism, as the efficiency is improved by approximately ω^2/Γ_D^2 (~10^6 for typical parameters) relative to the case of inductively coupled nonresonant objects.

Theoretical model for self-resonant coils. Our experimental realization of the scheme consists of two self-resonant coils. One coil (the source coil) is coupled inductively to an oscillating circuit; the other (the device coil) is coupled inductively to a resistive load (*12*) (Fig. 1). Self-resonant coils rely on the interplay between distributed inductance and distributed capacitance to achieve resonance. The coils are made of an electrically conducting wire of total length l and cross-sectional radius a wound into a helix of n turns, radius r, and height h. To the best of our knowledge, there is no exact solution for a finite helix in the literature, and even in the case of infinitely long coils, the solutions rely on assumptions that are inadequate for our system (*13*). We have found, however, that the simple quasi-static model described below is in good agreement (within ~5%) with experiment.

We start by observing that the current must be zero at the ends of the coil, and we make the educated guess that the resonant modes of the coil are well approximated by sinusoidal current profiles along the length of the conducting wire. We are interested in the lowest mode, so if we denote by s the parameterization coordinate along the length of the conductor, such that it runs from $-l/2$ to $+l/2$, then the time-dependent current profile has the form $I_0 \cos(\pi s/l)\exp(i\omega t)$. It follows from the continuity equation for charge that the linear charge density profile is of the form $\lambda_0 \sin(\pi s/l)\exp(i\omega t)$, so that one-half of the coil (when sliced perpendicularly to its axis) contains an oscillating total charge (of amplitude $q_0 = \lambda_0 l/\pi$) that is equal in magnitude but opposite in sign to the charge in the other half.

As the coil is resonant, the current and charge density profiles are $\pi/2$ out of phase from each

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other, meaning that the real part of one is maximum when the real part of the other is zero. Equivalently, the energy contained in the coil is at certain points in time completely due to the current, and at other points it is completely due to the charge. Using electromagnetic theory, we can define an effective inductance L and an effective capacitance C for each coil as follows:

$$L = \frac{\mu_0}{4\pi|I_0|^2}\iint d\mathbf{r}d\mathbf{r}'\frac{\mathbf{J}(\mathbf{r})\cdot\mathbf{J}(\mathbf{r}')}{|\mathbf{r}-\mathbf{r}'|} \quad (3)$$

$$\frac{1}{C} = \frac{1}{4\pi\varepsilon_0|q_0|^2}\iint d\mathbf{r}d\mathbf{r}'\frac{\rho(\mathbf{r})\rho(\mathbf{r}')}{|\mathbf{r}-\mathbf{r}'|} \quad (4)$$

where the spatial current $\mathbf{J}(\mathbf{r})$ and charge density $\rho(\mathbf{r})$ are obtained respectively from the current and charge densities along the isolated coil, in conjunction with the geometry of the object. As defined, L and C have the property that the energy U contained in the coil is given by

$$U = \frac{1}{2}L|I_0|^2 = \frac{1}{2C}|q_0|^2 \quad (5)$$

Given this relation and the equation of continuity, the resulting resonant frequency is $f_0 = 1/[2\pi(LC)^{1/2}]$. We can now treat this coil as a standard oscillator in coupled-mode theory by defining $a(t) = [(L/2)^{1/2}]I_0(t)$.

We can estimate the power dissipated by noting that the sinusoidal profile of the current distribution implies that the spatial average of the peak current squared is $|I_0|^2/2$. For a coil with n turns and made of a material with conductivity σ, we modify the standard formulas for ohmic (R_o) and radiation (R_r) resistance accordingly:

$$R_o = \sqrt{\frac{\mu_0\omega}{2\sigma}}\frac{l}{4\pi a} \quad (6)$$

$$R_r = \sqrt{\frac{\mu_0}{\varepsilon_0}}\left[\frac{\pi}{12}n^2\left(\frac{\omega r}{c}\right)^4 + \frac{2}{3\pi^3}\left(\frac{\omega h}{c}\right)^2\right] \quad (7)$$

The first term in Eq. 7 is a magnetic dipole radiation term (assuming $r << 2\pi c/\omega$, where c is the speed of light); the second term is due to the electric dipole of the coil and is smaller than the first term for our experimental parameters. The coupled-mode theory decay constant for the coil is therefore $\Gamma = (R_o + R_r)/2L$, and its quality factor is $Q = \omega/2\Gamma$.

We find the coupling coefficient κ_{DS} by looking at the power transferred from the source to the device coil, assuming a steady-state solution in which currents and charge densities vary in time as $\exp(i\omega t)$:

$$\begin{aligned} P_{DS} &= \int d\mathbf{r}\mathbf{E}_S(\mathbf{r})\cdot\mathbf{J}_D(\mathbf{r}) \\ &= -\int d\mathbf{r}\left[\dot{\mathbf{A}}_S(\mathbf{r}) + \nabla\phi_S(\mathbf{r})\right]\cdot\mathbf{J}_D(\mathbf{r}) \\ &= -\frac{1}{4\pi}\iint d\mathbf{r}d\mathbf{r}' \\ &\times\left[\mu_0\frac{\dot{\mathbf{J}}_S(\mathbf{r}')}{|\mathbf{r}'-\mathbf{r}|} + \frac{\rho_S(\mathbf{r}')}{\varepsilon_0}\frac{\mathbf{r}'-\mathbf{r}}{|\mathbf{r}'-\mathbf{r}|^3}\right]\cdot\mathbf{J}_D(\mathbf{r}') \\ &= -i\omega M I_S I_D \end{aligned} \quad (8)$$

Where M is the effective mutual inductance, ϕ is the scalar potential, $\mathbf{A}$ is the vector potential, and the subscript S indicates that the electric field is due to the source. We then conclude from standard coupled-mode theory arguments that $\kappa_{DS} = \kappa_{SD} = \kappa = \omega M/[2(L_S L_D)^{1/2}]$. When the distance D between the centers of the coils is much larger than their characteristic size, κ scales with the D^{-3} dependence characteristic of dipole-dipole coupling. Both κ and Γ are functions of the frequency, and κ/Γ and the efficiency are maximized for a particular value of f, which is in the range 1 to 50 MHz for typical parameters of interest. Thus, picking an appropriate frequency for a given coil size, as we do in this experimental demonstration, plays a major role in optimizing the power transfer.

Comparison with experimentally determined parameters. The parameters for the two identical helical coils built for the experimental validation of the power transfer scheme are h = 20 cm, a = 3 mm, r = 30 cm, and n = 5.25. Both coils are made of copper. The spacing between loops of the helix is not uniform, and we encapsulate the uncertainty about their uniformity by attributing a 10% (2 cm) uncertainty to h. The expected resonant frequency given these dimensions is f_0 = 10.56 ± 0.3 MHz, which is about 5% off from the measured resonance at 9.90 MHz.

The theoretical Q for the loops is estimated to be ~2500 (assuming $\sigma = 5.9 \times 10^7$ m/ohm), but the measured value is Q = 950 ± 50. We believe the discrepancy is mostly due to the effect of the layer of poorly conducting copper oxide on the surface of the copper wire, to which the current is confined by the short skin depth (~20 μm) at this frequency. We therefore use the experimentally observed Q and $\Gamma_S = \Gamma_D = \Gamma = \omega/2Q$ derived from it in all subsequent computations.

We find the coupling coefficient κ experimentally by placing the two self-resonant coils (fine-tuned, by slightly adjusting h, to the same resonant frequency when isolated) a distance D apart and measuring the splitting in the frequencies of the two resonant modes. According to coupled-mode theory, this splitting should be $\Delta\omega = 2[(\kappa^2 - \Gamma^2)^{1/2}]$. In the present work, we focus on the case where the two coils are aligned coaxially (Fig. 2), although similar results are obtained for other orientations (figs. S1 and S2).

Measurement of the efficiency. The maximum theoretical efficiency depends only on the parameter $\kappa/[(L_S L_D)^{1/2}] = \kappa/\Gamma$, which is greater than 1 even for D = 2.4 m (8 times the radius of the coils) (Fig. 3). Thus, we operate in the strongly coupled regime throughout the entire range of distances probed.

As our driving circuit, we use a standard Colpitts oscillator whose inductive element consists of a single loop of copper wire 25 cm in radius (Fig. 1); this loop of wire couples inductively to the source coil and drives the entire wireless power transfer apparatus. The load consists of a calibrated light bulb (*14*) and is attached to its own loop of insulated wire, which is placed in proximity of the device coil and inductively coupled to it. By varying the distance between the light bulb and the device coil, we are able to adjust the parameter Γ_W/Γ so that it matches its optimal value, given theoretically by $[1 + (\kappa^2/\Gamma^2)]^{1/2}$. (The loop connected to the

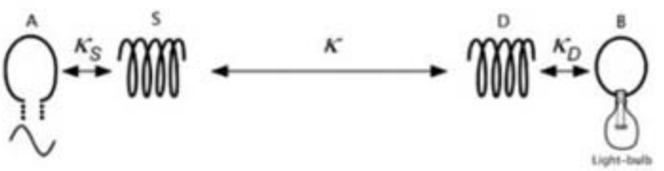

Fig. 1. Schematic of the experimental setup. A is a single copper loop of radius 25 cm that is part of the driving circuit, which outputs a sine wave with frequency 9.9 MHz. S and D are respectively the source and device coils referred to in the text. B is a loop of wire attached to the load (light bulb). The various κs represent direct couplings between the objects indicated by the arrows. The angle between coil D and the loop A is adjusted to ensure that their direct coupling is zero. Coils S and D are aligned coaxially. The direct couplings between B and A and between B and S are negligible.

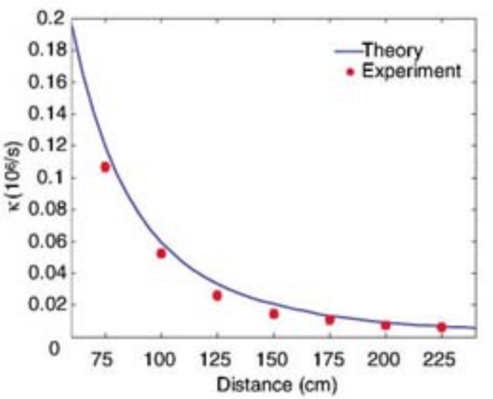

Fig. 2. Comparison of experimental and theoretical values for κ as a function of the separation between coaxially aligned source and device coils (the wireless power transfer distance).

light bulb adds a small reactive component to Γ_W, which is compensated for by slightly retuning the coil.) We measure the work extracted by adjusting the power going into the Colpitts oscillator until the light bulb at the load glows at its full nominal brightness.

We determine the efficiency of the transfer taking place between the source coil and the load by measuring the current at the midpoint of each of the self-resonant coils with a current probe (which does not lower the Q of the coils noticeably). This gives a measurement of the current parameters I_S and I_D used in our theoretical model. We then compute the power dissipated in each coil from $P_{S,D} = \Gamma L |I_{S,D}|^2$, and obtain the efficiency from $\eta = P_W/(P_S + P_D + P_W)$. To ensure that the experimental setup is well described by a two-object coupled mode theory model, we position the device coil such that its direct coupling to the copper loop attached to the Colpitts oscillator is zero. The experimental results are shown in Fig. 4, along with the theoretical prediction for maximum efficiency, given by Eq. 2. We were able to transfer several tens of watts with the use of this setup, fully lighting up a 60-W light bulb from distances more than 2 m away (figs. S3 and S4).

As a cross-check, we also measured the total power going from the wall power outlet into the driving circuit. The efficiency of the wireless transfer itself is hard to estimate in this way, however, as the efficiency of the Colpitts oscillator itself is not precisely known, although it is expected to be far from 100% (*15*). Still, the ratio of power extracted to power entering the driving circuit gives a lower bound on the efficiency. When transferring 60 W to the load over a distance of 2 m, for example, the power flowing into the driving circuit is 400 W. This yields an overall wall-to-load efficiency of 15%, which is reasonable given the expected efficiency of 40 to 50% for the wireless power transfer at that distance and the low efficiency of the Colpitts oscillator.

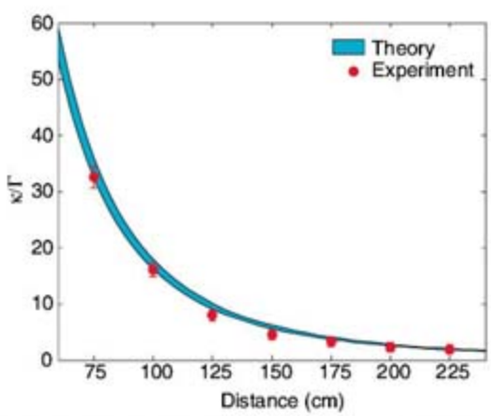

Fig. 3. Comparison of experimental and theoretical values for the parameter κ/Γ as a function of the wireless power transfer distance. The theory values are obtained by using the theoretical κ and the experimentally measured Γ. The shaded area represents the spread in the theoretical κ/Γ due to the 5% uncertainty in Q.

Concluding remarks. It is essential that the coils be on resonance for the power transfer to be practical (*6*). We find experimentally that the power transmitted to the load drops sharply as either one of the coils is detuned from resonance. For a fractional detuning $\Delta f/f_0$ of a few times the inverse loaded Q, the induced current in the device coil is indistinguishable from noise.

A detailed and quantitative analysis of the effect of external objects on our scheme is beyond the scope of this work, but we note here that the power transfer is not visibly affected as humans and various everyday objects, such as metals, wood, and electronic devices large and small, are placed between the two coils—even in cases where they completely obstruct the line of sight between source and device (figs. S3 to S5). External objects have a noticeable effect only when they are within a few centimeters from either one of the coils. Some materials (such as aluminum foil, Styrofoam, and humans) mostly just shift the resonant frequency, which can in principle be easily corrected with a feedback circuit; other materials (cardboard, wood, and polyvinyl chloride) lower Q when placed closer than a few centimeters from the coil, thereby lowering the efficiency of the transfer.

When transferring 60 W across 2 m, we calculate that at the point halfway between the coils, the root mean square (RMS) magnitude of the electric field is $E_{RMS} = 210$ V/m, that of the magnetic field is $H_{RMS} = 1$ A/m, and that of the Poynting vector is $S_{RMS} = 3.2$ mW/cm^2 (*16*). These values increase closer to the coils, where the fields at source and device are comparable. For example, at distances 20 cm away from the surface of the device coil, we calculate the maximum values for the fields to be $E_{RMS} = 1.4$ kV/m, $H_{RMS} = 8$ A/m, and $S_{RMS} = 0.2$ W/cm^2. The power radiated for these parameters is ~5 W, which is roughly an order of magnitude higher than cell phones. In the particular geometry that we studied, the overwhelming contribution (by one to two orders of magnitude) to the electric near-field, and hence to the near-field Poynting vector, comes from the electric dipole moment of the coils. If instead one uses a capacitively loaded single-turn loop design (*6*)—which has the advantage of confining nearly all of the electric field inside the capacitor—and tailors the system to operate at lower frequencies, our calculations show (*17*) that it should be possible to reduce the values cited above for the electric and magnetic fields, the Poynting vector, and the power radiated so that they fall below thresholds specified by general safety regulations [e.g., the IEEE safety standards for general public exposure (*18*)].

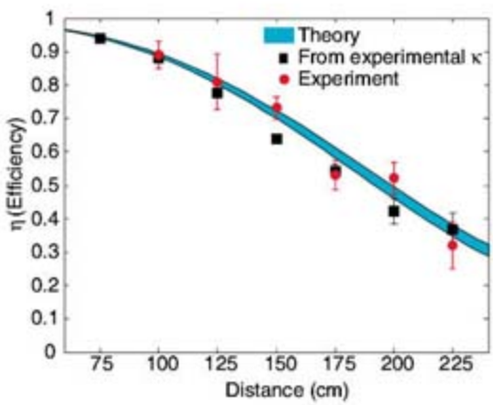

Fig. 4. Comparison of experimental and theoretical efficiencies as functions of the wireless power transfer distance. The shaded area represents the theoretical prediction for maximum efficiency and is obtained by inserting the theoretical values from Fig. 3 into Eq. 2, with $\Gamma_W/\Gamma_D = [1 + (\kappa^2/\Gamma^2)]^{1/2}$. The black squares are the maximum efficiency obtained from Eq. 2 and the experimental values of κ/Γ from Fig. 3. The red dots present the directly measured efficiency, as described in the text.

Although the two coils are currently of identical dimensions, it is possible to make the device coil small enough to fit into portable devices without decreasing the efficiency. One could, for instance, maintain the product of the characteristic sizes of the source and device coils constant, as argued in (*6*).

We believe that the efficiency of the scheme and the power transfer distances could be appreciably improved by silver-plating the coils, which should increase their Q, or by working with more elaborate geometries for the resonant objects (*19*). Nonetheless, the performance characteristics of the system presented here are already at levels where they could be useful in practical applications.

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Figs. S1 to S5

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Sea Anemone Genome Reveals Ancestral Eumetazoan Gene Repertoire and Genomic Organization

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Sea anemones are seemingly primitive animals that, along with corals, jellyfish, and hydras, constitute the oldest eumetazoan phylum, the Cnidaria. Here, we report a comparative analysis of the draft genome of an emerging cnidarian model, the starlet sea anemone *Nematostella vectensis*. The sea anemone genome is complex, with a gene repertoire, exon-intron structure, and large-scale gene linkage more similar to vertebrates than to flies or nematodes, implying that the genome of the eumetazoan ancestor was similarly complex. Nearly one-fifth of the inferred genes of the ancestor are eumetazoan novelties, which are enriched for animal functions like cell signaling, adhesion, and synaptic transmission. Analysis of diverse pathways suggests that these gene "inventions" along the lineage leading to animals were likely already well integrated with preexisting eukaryotic genes in the eumetazoan progenitor.

All living tissue-grade animals, or eumetazoans, are descended from the last common ancestor of bilaterians (flies, worms, snails, and humans), cnidarians (anemones, jellyfish, and hydra), and ctenophores (comb jellies) (*1*, *2*). This eumetazoan ancestor lived perhaps 700 million years ago. Although it is not preserved in the fossil record (*3*), we can infer many of its characteristics—flagellated sperm, development through a process of gastrulation, multiple germ layers, true epithelia lying upon a basement membrane, a lined gut (enteron), a neuromuscular system, multiple sensory systems, and fixed body axes—because these conserved features are retained by its modern descendants.

Similarly, we can characterize the genome of this long-extinct eumetazoan progenitor by comparing modern DNA and protein sequences and identifying conserved ancestral features that have an intrinsically slow rate of change and/or are preserved by selective pressures. Comparisons (*4*–*6*) between fruit fly, nematode, and vertebrate genomes reveal greater genomic complexity in the vertebrates [and other deuterostomes (*7*, *8*)] as measured by gene content and structure, but at the same time show that many genes and networks are shared across bilaterians. Probing the ancestral eumetazoan genome requires sequences from even deeper branches of the animal tree, comparing bilaterian and nonbilaterian phyla.

In comparison with bilaterians, cnidarians appear morphologically simple. The phylum is defined (*2*) by a sac-like body plan with a single oral opening, two epithelial tissue layers, the presence of numerous tentacles, a nerve net, and the characteristic stinging cells (cnidocytes, literally translated as "nettle cells") that give the phylum its name (fig. S1.1G). The class Anthozoa ("flower animals") includes diverse anemones, corals, and sea pens, all of which lack a medusa stage. The other cnidarian classes are united by their pelagic medusae and characteristically linear mitochondrial genomes (*9*) into the Medusozoa, including *Hydra* and related hydroids, jellyfish, and box jellies. The disparate bilaterian phyla of the early Cambrian suggest a Precambrian divergence of the cnidarian lineage from the bilaterian stem, and indeed some of the oldest animal body and embryo fossils are plausibly relics of stem cnidarians [reviewed in (*10*, *11*)].

Among Anthozoan cnidarians, the starlet sea anemone *Nematostella vectensis* is an emerging model system (*12*, *13*). This estuarine burrowing anemone is found on the Atlantic and Pacific coasts of North America, as well as the coast of southeast England (*14*). *Nematostella* cultures are easily maintained in the laboratory; with separate sexes, inducible spawning, and external fertilization (*12*, *15*), embryos are available throughout the year.

Although cnidarians are often characterized as simple or primitive, closer study of *Nematostella* and its relatives has revealed considerable molecular (*16*–*19*) and morphological complexity (*13*). Based on expressed sequence tag (EST) analyses (*17*, *18*) and the targeted study of specific gene families [reviewed in (*13*, *16*, *20*–*22*)], signaling pathways and transcription factors involved in the early patterning and development of bilaterians are present in cnidarian genomes and are active in development (*13*, *23*–*28*), indicating that these pathways and regulatory mechanisms predate the eumetazoan radiation. Perhaps most notably, genes that establish the main body axes in bilaterian embryos are also expressed asymmetrically in *Nematostella* development, even though cnidarians are conventionally viewed as radial animals [for a critical discussion, see (*29*)].

Here, we report the draft genome of the starlet sea anemone and use its gene repertoire and genome organization to reconstruct features of the ancestral eumetazoan genome. Analysis of the *Nematostella* genome in the context of sequences from other eukaryotes reveals the genomic complexity of this last common cnidarian-bilaterian ancestor. The emerging picture from the genome and EST studies (*17*, *18*) is one of extensive conservation in gene content, structure, and organization between *Nematostella* and vertebrates. We show that even chromosome-scale linkage has been preserved between *Nematostella* and vertebrates. These are the most ancient conserved linkages known outside of prokaryotic operons. In contrast, the fruit fly and nematode model systems have experienced extensive gene loss (*18*), intron loss (*30*), and genome rearrangement. Thus, from a genomic perspective, the eumetazoan ancestor more closely resembled modern vertebrates and sea anemones.

Nematostella Genome Assembly and Gene Set

The draft sequence of the *Nematostella* genome was produced with the use of a random shotgun

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strategy (*31*) from approximately 6.5-fold redundant paired-end sequence coverage from several shotgun libraries of a range of insert sizes derived from a single mating pair with ~0.8% allelic variation. [For a detailed discussion of polymorphism, see supporting online material (SOM) text (*32*)]. The total assembly spans ~357 megabases (Mb), with half of this sequence in 181 scaffolds longer than ~470 kb. Metaphase spreads indicate a diploid chromosome number of $2n = 30$ (fig. S2.4). Currently, there are no physical or genetic maps of *Nematostella*, so we could not reconstruct the genome as chromosomes. Nevertheless, because half of the predicted genes are in scaffolds containing 48 or more genes, the present draft assembly is sufficiently long-range to permit useful analysis of synteny with other species. The typical locus in the draft genome is in a contiguous gap-free stretch of nearly 20 kb. Comparison of the assembled sequence with open reading frames derived from ESTs shows that the assembly captures ~95% of the known protein-coding content (*32*). Although approximately one-third of the shotgun sequences were not assembled, they could typically be characterized as derived from long (>100 kb) tandem-repetitive minisatellite arrays suggestive of heterochromatin, implying a total genome size of ~450 Mb (*32*).

We estimated that the *Nematostella* genome contains ~18,000 bona fide protein-coding genes, comparable to gene counts in other animals. Combining homology-based and ab initio methods with sequences from more than 146,000 ESTs, we predicted ~27,000 complete or partial protein-coding transcripts in the genome (*32*). More than 12,000 of these are found in robust eumetazoan gene families and are therefore supported as orthologs of genes in other animals. Whereas ~22,000 of all predicted genes have a significant alignment [Basic Local Alignment Search Tool (BLAST) *e* value $< 10^{-10}$] to known proteins in SwissProt/Trembl and therefore have some homology support, analysis of a random sampling of genes suggests that some of these appear to be gene fragments, possible pseudogenes, relics of transposable elements, or allelic variants, leading to a discounting of the true gene count to ~18,000 (*32*). More than 25% of the genome is composed of repetitive elements that are mutated inactive transposable elements, including DNA transposons and both long terminal repeat (LTR) and non-LTR retrotransposons (table S2.3).

The Ancestral Eumetazoan Gene Set

By comparing the gene complement of *Nematostella* with other metazoans, we attempted to reconstruct the gene repertoire of the eumetazoan (i.e., cnidarian-bilaterian) ancestor and to infer the gains, losses, and duplications that occurred both before and after the eumetazoan radiation. To approximate the gene repertoire of the eumetazoan ancestor, we constructed 7766 putatively orthologous gene families that are anchored by reciprocal best-scoring BLAST alignments (*33*) between genes from anemone and one or more of fly, nematode, human, frog, or pufferfish (*32*). Each family thus represents a single gene in the eumetazoan ancestor whose descendants survive in recognizable form as modern genes in both cnidarians and bilaterians. These families account for a substantial fraction of genes in modern animals: We estimated that nearly two-thirds of human genes (13,830) are descended from these progenitors through subsequent gene family expansions along the human lineage, and a comparable number (12,319) of predicted *Nematostella* genes arose by independent diversifications along the cnidarian branch, but only 7309 (~50%) and 7261 (~40%) were found in *Drosophila* and *Caenorhabditis elegans*, respectively. Given that we cannot capture genes that were present in the eumetazoan progenitor but became highly diverged or lost in one or more sequenced descendants, our reconstructed ancestral gene set is necessarily incomplete, but it nevertheless provides a starting point for further analysis.

Of the 7766 ancestral eumetazoan gene families, only 72% (5626) are represented in the complete genomes of all three major modern eumetazoan lineages: cnidarians (i.e., *Nematostella*), protostomes (i.e., *Drosophila* and/or *C. elegans*), and deuterostomes (requiring presence of at least two of pufferfish, frog, and human). We found 1292 eumetazoan gene families that had detectable descendants in anemone and at least two of the three vertebrates, but that appeared to be absent in both fruit fly and soil nematode. This indicates that they were either lost or highly diverged in both of these model protostomes, extending the list of such genes found in EST studies (*17*, *18*). The forthcoming genome sequences of crustaceans, annelids, and mollusks will help address which of these genes survived in the protostome lineage but were convergently lost in flies and nematodes. In contrast, only 33 genes were found in *Nematostella* and both *Drosophila* and *C. elegans*, but not in any vertebrate. These results represent putative deuterostome or vertebrate loss, indicating a much lower degree of gene loss in the vertebrates than in the ecdysozoan model systems. We found 673 gene families that were represented in model protostomes and vertebrates but not in *Nematostella*. These are candidates for bilaterian novelties, but some will no doubt turn out to be losses or divergent sequences in *Nematostella*.

Molecular Evolution of the Eumetazoa

To address evolutionary relationships between animals, we inferred the phylogeny of Metazoa by combining *Nematostella* data with available genomic sequences from diverse animals, using a subset of 337 single-copy genes suitable for deep phylogenetic analysis (*32*). In Fig. 1, relative branch lengths represent the accumulation of amino acid substitutions in each lineage across this set of proteins. Our whole-genome analysis groups the fruit fly with the soil nematode, in support of the superphylum Ecdysozoa, a major element of the "new animal phylogeny" (*34*), in contrast with other whole-genome–based studies that support an early branching acoelomate clade that includes *C. elegans* (*35*, *36*). As expected,

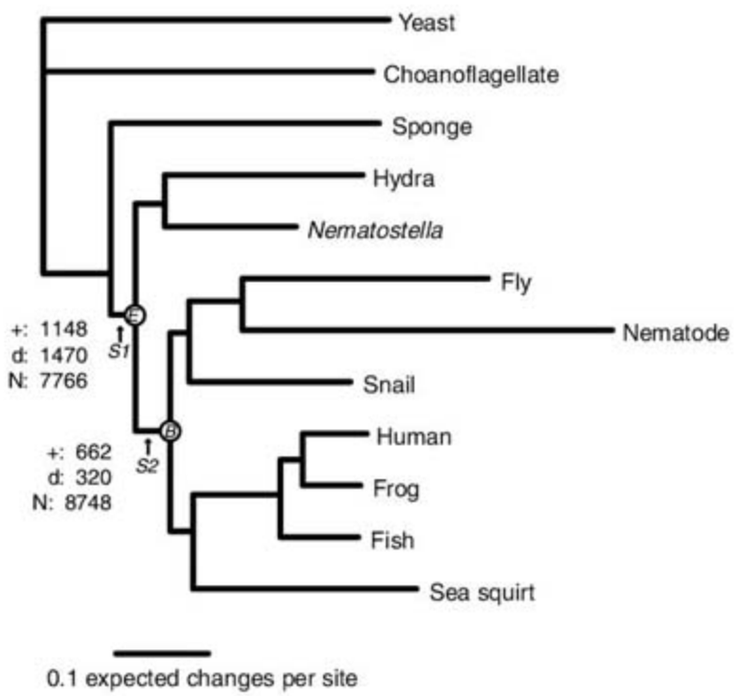


Fig. 1. Bayesian phylogeny of Metazoa. Bayesian analysis infers metazoan phylogeny and rate of amino acid substitution from sequenced genomes based on 337 single-copy genes in *Ciona intestinalis* (sea squirt), *Takifugu rubripes* (fish), *Xenopus tropicalis* (frog), human, *Lottia gigantea* (snail), *Drosophila melanogaster* (fly), *C. elegans* (nematode), *Hydra magnipapillata* (hydra), *Nematostella*, *Amphimedon queenslandica* (sponge), *Monosiga brevicollis* (choanoflagellate), and *Saccharomyces cerevisiae* (yeast). All nodes were resolved as shown in 100% of sampled topologies in Bayesian analysis. The scale bar indicates the expected number of amino acid substitutions per aligned amino acid position. E, the eumetazoan (cnidarian-bilaterian) ancestor; B, the bilaterian (protostome-deuterostome) ancestor. The number of new genes (+), genes created by gene duplication (d), and the total number of reconstructed ancestral genes of the recent common ancestor (N) are labeled for S1 and S2, the eumetazoan and bilaterian stems, respectively (*32*).

the two cnidarians *Nematostella* and *Hydra* form a monophyletic group that branched off the metazoan stem before the radiation of bilaterians. The depth of the *Nematostella-Hydra* split (comparable to the protostome-deuterostome divergence) emphasizes the distant relationship between anthozoans and hydrozoans. This supports the paleontological evidence that the radiation of the cnidarian phylum is quite ancient (*37*) and suggests that substantial variation in gene content and gene-family diversity may be found when the anemone genome is compared with that of the hydrozoan *Hydra*. For convenience, here we refer to the last common ancestor of cnidarians and bilaterians as the eumetazoan ancestor, although the precise phylogenetic placement of ctenophores may revise this designation.

Long branch lengths, indicating increased levels of sequence divergence, were found along the fly, nematode, and sea squirt lineages, consistent with systematic trends observed in BLAST-based analyses of ESTs (*17*, *18*). The sea anemone sequences, however, appear to be evolving at a rate comparable to, or even somewhat slower than, vertebrates. Although accelerated rates of molecular evolution have been documented in flies and echinoderms (*38*) relative to vertebrates, our analysis does not support the extrapolation of these higher rates to all invertebrates. With the use of our branch lengths, a very crude molecular clock interpolation based on the eukaryotic time scales of Douzery *et al.* (*39*) suggests that the eumetazoan ancestor lived ~670 to 820 million years ago (*32*). This very rough estimate has numerous caveats—most notably that there is no guarantee that the rate of protein evolution was constant on the eumetazoan stem—but provides a rough time scale for the eumetazoan radiation.

Conservation of Ancient Eumetazoan Introns

Comparison of *Nematostella* genes to those of other animals reveals that the ancestral eumetazoan genome must have been intron-rich, with gene structures closely resembling those of modern vertebrate and anemone genes. Introns that are shared between *Nematostella* and vertebrates and/or other bilaterians are most parsimoniously interpreted as conserved ancient eumetazoan introns (*40*). Not only are the numbers of exons per gene similar between *Nematostella* and vertebrates, but the precise location and phase (i.e., the positioning of the splice sites relative to codon boundaries) of introns are also highly conserved between the anemone and human (Fig. 2A). Within alignable regions, nearly 81% of human introns are found in the same position and phase in *Nematostella*; conversely, 82% of the anemone introns are found in orthologous positions in human genes (*32*). Whereas intron conservation between the annelid *Platynereis* and vertebrates implies that the Protostome-Deuterostome ancestor was intron-rich (*30*), the analysis of *Nematostella* extends this result to the eumetazoan ancestor.

Using whole-genome data sets, we estimated the tempo of intron evolution across metazoan genomes (*32*). Figure 2B shows intron gain and loss events inferred by weighted parsimony analysis of 2645 intron positions that lie within highly conserved protein sequence in five representative animals, the flowering plant *Arabidopsis*, and the relatively intron-rich fungus *Cryptococcus neoformans* (*32*). Although fungi and animals are phylogenetically closer to each other than either group is to plants, fungi are not by themselves a sufficient outgroup for characterizing the history of eumetazoan introns, given that there are putative ancient eukaryotic introns shared by modern animals and plants that have evidently been lost in fungi (*41*).

Although many eumetazoan introns are evidently of ancient eukaryotic origin (*41*)—for example, nearly 26% of human and *Nematostella* introns are conserved with *Arabidopsis*, and 24% with *Cryptococcus*—the remainder appear to be shared only by animals. These animal introns are most parsimoniously accounted for as gains on the eumetazoan stem, as shown by the long "gain" branch in Fig. 2B. We cannot rule out the possibility, however, that such apparently animal-specific introns were indeed present in the last common ancestor of plants, fungi, and animals, but were convergently lost in both plants and fungi. Within animals, intron gains range from 8 to 22% relative to the content of the eumetazoan ancestor. Thus, assuming ~8 introns per ancestral gene, ~1 novel intron has been introduced in a typical

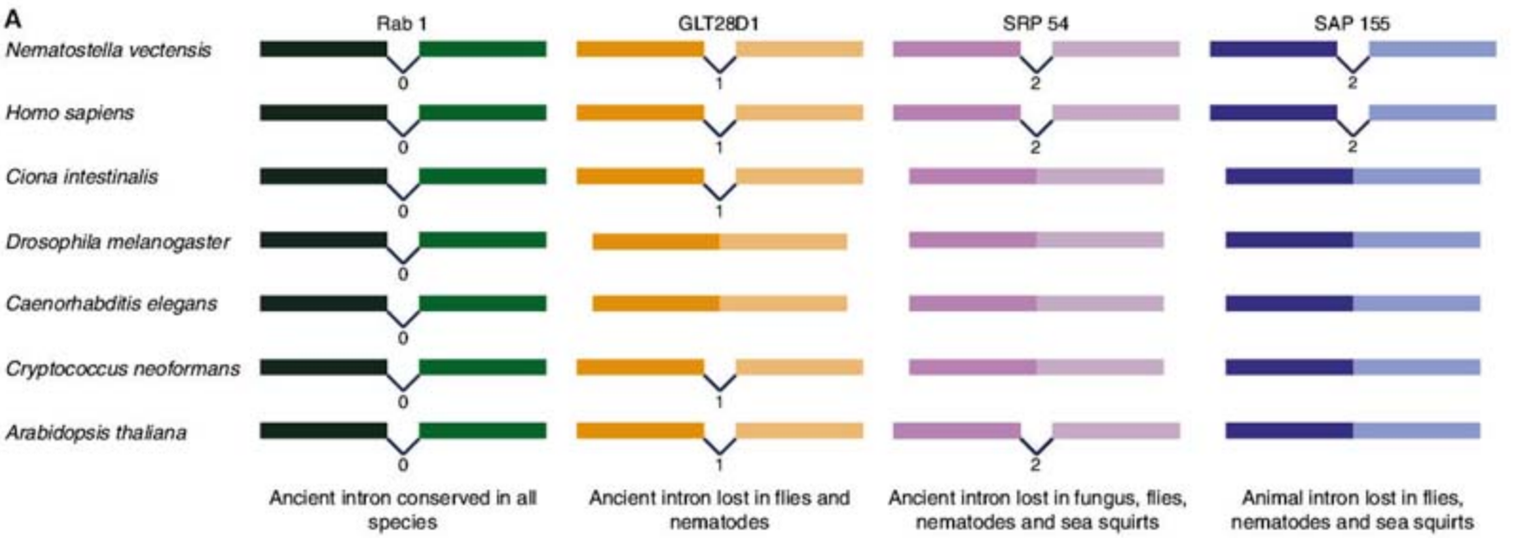
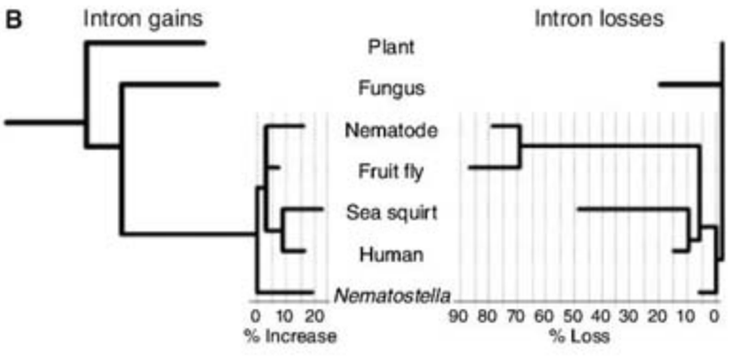


Fig. 2. Patterns of intron evolution in eukaryotes. (**A**) Examples of different patterns of intron gain and loss. Bars of the same color represent conserved regions across all species. Chevrons indicate introns and the number below the chevron shows the phase of the intron. (**B**) Branch lengths proportional to the number of inferred intron gains (left), and intron losses (right) under the Dollo parsimony assumption that introns with conserved position and phase were gained only once in evolution. The bottom scale indicates the change in intron number for gains (left) and losses (right), relative to the inferred introns of the eumetazoan ancestor. Based on a sample of 5175 introns at highly conserved protein sequence positions from *Arabidopsis thaliana* (plant), *Cryptococcus neoformans* (fungus), *C. elegans* (nematode), *D. melanogaster* (fly), *C. intestinalis* (sea squirt), *Homo sapiens* (human), and *Nematostella* (*32*).



modern animal gene since the eumetazoan radiation, a rate of approximately ~10^{-9} introns per gene per year, which is comparable to the rate of gene duplication per locus per year (*42*).

In contrast to intron gains, which seem to occur more or less uniformly across animal phyla, some lineages appear to have experienced extensive intron loss, notably the fly, nematode, and sea squirt, which have each discarded 50 to 90% of inferred ancestral eumetazoan introns. It remains to be seen whether the introns absent in both fly and nematode are the result of ancient loss in the ecdysozoan stem lineage (the most parsimonious explanation, shown in Fig. 2B) or are convergent (independent) losses in flies and nematodes. We can rule out ancient loss on the protostome stem on the basis of the results of Raible *et al.* (*30*) for the annelid *Platynereis*, which showed that the ancestral protostome genome was intron-rich.

Conservation of Ancient Eumetazoan Linkage Groups

Conserved linkage groups representing ancestral vertebrate chromosomes can be defined by comparing fish and mammalian genomes and genetic maps, despite the presence of only modest segments of conserved gene order (*43*, *44*). Similarly, limited conservation of synteny is recognizable within insects [such as between flies and bees (*45*)]. Between animal phyla, however, no large-scale conserved synteny has been identified, suggesting that signals of the ancestral eumetazoan genome organization were erased by subsequent chromosomal breaks and translocations along the various lineages. Despite extensive local scrambling of gene order, we find extensive conservation of synteny between the *Nematostella* and vertebrate genomes, allowing the identification of ancient eumetazoan linkage groups.

Reasoning that the prevalence of intrachromosomal inversions and rearrangements (*46*) might scramble local gene order yet preserve linkage, we searched for large-scale conserved synteny—that is, sets of orthologous genes on the same chromosomal segment in their respective genomes, regardless of gene order. To remove confounding signals from recent rearrangements, we used comparisons with the genomes of other chordates to identify 98 human segments that do not appear to have undergone recent breaks or fusions (Fig. 3A and fig. S7.1) (*32*). These segments span 89% of the human genome. The human genome was selected as a reference because it is known to have a slow rate of chromosome evolution relative to other mammals (*46*) and has preserved chromosomal segments relative to teleost fish (*43*). To search for ancient conserved linkages across eumetazoa, we then compared these human genome segments to the assembled *Nematostella* scaffolds, using a statistical test for distinguishing significant enrichment for genes linked in both species.

For every scaffold-segment pair, we tabulated the number of predicted ancestral eumetazoan genes with descendants found in both the *Nematostella* scaffold and human segment. This number of shared orthologous genes was compared to a null model in which the scaffolds and segments have gene content independently drawn from the ancestral set. The "Oxford grid" shown in Table 1 shows not only that there are many scaffold-segment pairs with a significant excess of shared ancestral genes, but that the anemone scaffolds and human chromosome segments can be grouped into classes, such that scaffold-segment pairs drawn from the same class are likely to have a significant excess of shared ancestral genes (*32*). Each class is most easily interpreted as collecting together segments of the present-day *Nematostella* and human genomes that descend from the same chromosome of the eumetazoan ancestor, and therefore defines a putative ancestral eumetazoan linkage group (PAL). The complete Oxford grid showing all 13 eumetazoan PALs is shown in table S7.2.

The conserved linkage is extensive, and it accounts for a large fraction of the ancestral eumetazoan set. Of the 4402 ancestral eumetazoan gene families represented in the largest anemone scaffolds and human segments (i.e., in the genomic regions large enough to permit statistically significant analysis and therefore eligible for consideration in our analysis), more than 30% (1336) participate in a conserved linkage group. This is a lower bound on the true extent of the remnant ancient linkage groups because the length of the *Nematostella* scaffolds and the use of conservative statistical criteria limit our analysis. A more sensitive approach can

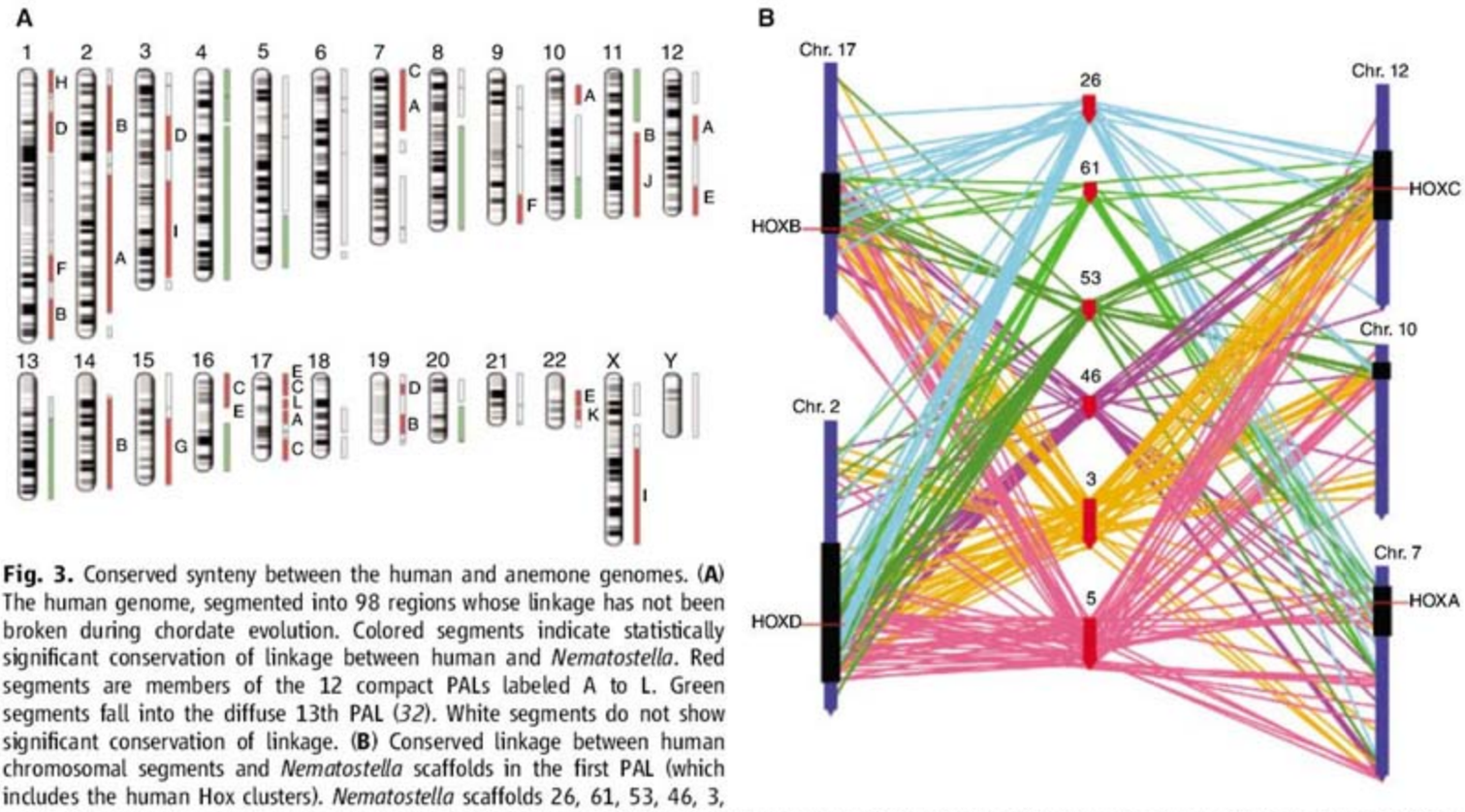


Fig. 3. Conserved synteny between the human and anemone genomes. (**A**) The human genome, segmented into 98 regions whose linkage has not been broken during chordate evolution. Colored segments indicate statistically significant conservation of linkage between human and *Nematostella*. Red segments are members of the 12 compact PALs labeled A to L. Green segments fall into the diffuse 13th PAL (*32*). White segments do not show significant conservation of linkage. (**B**) Conserved linkage between human chromosomal segments and *Nematostella* scaffolds in the first PAL (which includes the human Hox clusters). *Nematostella* scaffolds 26, 61, 53, 46, 3, and 5 (red arrows) and human chromosomes 17, 12, 10, 7, and 2 (blue arrows) are shown with length proportional to the number of genes descended from the inferred ancestral set. Lines, color-coded by *Nematostella* scaffold, join the positions of orthologous *Nematostella* and human genes. The five segments of the human genome that are grouped into PAL A are indicated by black boxes. Red lines indicate the positions of the four human Hox clusters.

assign more than twice as many ancestral genes to a PAL (*32*). The 40 human segments that show conserved synteny with *Nematostella* cover half of the human genome. Within such human segments, typically 40 to 50% of eumetazoan-derived genes have counterparts in syntenic *Nematostella* segments, and vice versa. This is a notable total, given that any chromosomal fusions and subsequent gene order scrambling on either the human or *Nematostella* lineage during their ~700 million years of independent evolution would attenuate the signal for linkage.

The observation of conserved linkage groups is most easily explained as the remnants of large ancestral chromosomal segments containing hundreds of genes that have evolved without obvious constraint on gene order within each block. Seven of the PALs link anemone scaffolds to multiple regions of the human genome in a manner consistent with multiple large-scale duplication events along the vertebrate lineage [reviewed in (*47*)]. These seven PALs represent the ancestral (preduplication) linkage of these regions. The extent of this conserved linkage suggests either that the neutral rate of interchromosomal translocations is low (on the order of a few breaks or fusions per chromosome since the eumetazoan ancestor, excluding intrachromosomal rearrangements) or that selection has acted to maintain linkage of large groups of genes, perhaps constrained by higher-level chromosomal organization (*48*) and/or long-range gene regulation (*49*).

An ancestral linkage group of particular interest includes the human Hox clusters of homeobox transcription factors that regulate anterior-posterior identity in bilaterians. Putative Hox genes in *Nematostella* and other cnidarians are also expressed in spatial patterns consistent with an ancient role in embryonic development (*50*–*52*). Tetrapods have four Hox clusters that arose by duplication on the vertebrate stem—HoxA (human chromosome 7p15.2), HoxB (17q21.32), HoxC (12q13.13), and HoxD (2q31.1)—which all appear in the same eumetazoan PAL, linked to eight *Nematostella* scaffolds (Fig. 3B), defining the ancestral genomic context for Hox genes. *Nematostella* has several clusters of homeobox genes (*52*–*54*), but only those on scaffolds 3 and 61 are embedded within the ancestral eumetazoan Hox context, providing independent support for the assignment of these homeobox genes as bona fide *Nematostella* Hox genes (*50*, *52*, *53*, *55*). There is an extensive block of 225 ancestral genes (table S7.3) that were linked to Hox in the eumetazoan ancestor and have retained that linkage in both the modern human and anemone genomes.

Origins of Eumetazoan Genes

Where did the eumetazoan gene repertoire come from? Nearly 80% (6182 out of 7766) of the ancestral eumetazoan genes have clearly identifiable relatives (i.e., proteins with significant sequence homology and conserved domain architecture) outside of the animals, including fungi, plants, slime molds, ciliates, or other species available from public data sets (*32*). These are evidently members of ancient eukaryotic gene families that were already established in the unicellular ancestors of the Metazoa and are involved in core eukaryotic cellular functions. Although these eumetazoan gene families are conserved with other eukaryotes, animals have a unique complement due to family expansion and contraction on the eumetazoan stem. The eumetazoan genes of ancient eukaryotic ancestry are themselves descended from ~5148 eukaryotic progenitors by nearly 1000 gene duplications along the eumetazoan stem—i.e., after the early radiation of eukaryotes ~1100 to 1500 million years ago (*56*) but before the divergence of cnidarians and bilaterians (*32*).

The remaining 20% (1584) of the ancestral eumetazoan gene set comprises animal novelties that were apparently "invented" along the eumetazoan stem. The mechanism for the creation of "new" genes is obscure (*57*) but may involve gene duplication followed by bursts of rapid sequence divergence (thus masking the similarity with sister sequences) and/or de novo recruitment of gene and/or noncoding fragments into functional transcription units. We classified these eumetazoan novelties into three categories based on their origin (Fig. 4A).

The first and largest group (type I novelty) comprises animal genes that have no identifiable relatives (with BLAST) outside of animals in the available sequence data sets, and accounts for 15% (1186) of ancestral eumetazoan genes. These include important signaling factors, such as the secreted wingless (Wnt) and fibroblast growth factor (FGF) families, and transcription factors, including the T-box and mothers-against-decapentaplegic (SMAD) families (Table 2). Not only were these genes present in the eumetazoan ancestor, but they had already duplicated and diversified on the eumetazoan stem to establish the subfamilies that, nearly 700 million years later, are still maintained in modern vertebrates. [See for example the Wnt family (*58*).]

Type II novelties (2% of the eumetazoan complement, or 158 genes) incorporate animal-only domains in combination with ancient eukaryotic sequence. The ancestry of these genes can be traced back to the eukaryotic radiation through their ancient domains, but the novel domains they contain were evidently invented (or evolved into their recognizable animal form) and coupled to more ancient domains on the eumetazoan stem. For example, Notch proteins have two Notch domains found only in metazoans in addition to ancient eukaryotic ankyrin and epidermal growth factor (EGF) domains; focal adhesion kinase (FAK) is targeted to focal adhesions in eumetazoans because of the addition of an animal-specific focal adhesion–targeting domain to the ancient kinase domain.

Table 1. Detail of the "Oxford grid" which tabulates the number of ancestral gene clusters shared between the 22 *Nematostella* scaffolds (columns) and 14 segments of the human genome (rows) that are assigned to PALs A, B and C. Cell symbols indicate Bonferroni-corrected *P* value < 0.01 (*), < 0.05 (†), < 0.5 (‡). Detailed methods, and the complete Oxford grid can be found in the SOM text.

PAL:		A								B								C					
Nematostella scaffold		3	5	46	26	53	61	44	144	7	74	18	88	52	42	156	89	10	8	34	118	91	191
Human Chromosome Segment	2q11.2-35	25*	32*	17*	16*	17*	9*	12*	7*	1	2			2	1		1	1	1	2			
	12q12-14.3	16*	14*	9*	5	8*	3	6‡	5†			1								1	1		
	17q12-21.32	12*	8‡	4	10*	6†	4	3	1			1	1		1				1				
A	7p11.2-21.3	4	10*	3	3	2	7*	1	2			1	1					2	1	1	1	1	
	10p11.22-13	8*	6†	1	1	2	1	4‡	1														
	14q12-32.33	10	3	2	4	5	3	3	2	23*	12*	13*	11*	17*	11*	9*	8*	1	2			1	
	11q12.1-13.1	4					2	2	1	12*	7*	1	6*	6†	1		4			2			
B	1q32.2-44	6	4		1	1	2		1	11*	6*	6‡	3	6†	2	2	1						1
	19q13.11-13.33	4	1	2	2	1	2	1		5	8*	8*	4	6‡	2		3	2	3		1	1	
	2p13.2-24.3	5	2	2	1	2	1	2	1	8‡	5	10*	5	3	1	5‡	3	2		1			
	17q23.3-25.3	1	2						1	2		2	1					19*	10*	12*	8*	7*	3
C	16p11.2-13.3	1			1	1						1	1	1				17*	19*	9*	5‡	6†	6*
	7p22.1-22.3											1		1	1			6*	3	3	5*	2	
	17p11.2-13.1				1						1			1			1	6*	2		1	3	

Finally, type III novelties (3%, or 240 gene families) consist of animal genes whose domains are all ancient (i.e., each found in other eukaryotes) but that occur in apparently unique combination in eumetazoa relative to known nonanimal genes (*32*) because of gene fusions and/or domain-shuffling events on the eumetazoan stem. For example, both the LIM (lin-11, islet, mec-3) protein-protein interaction and homeobox DNA binding domains are found in nonanimal eukaryotes, but only animals have the LIM-homeodomain combination. Although such domain-shuffling (*57*) events are relatively rare, they are disproportionately involved in characterized biochemical pathways, perhaps by bringing together existing catalytic capabilities, localization, and regulatory domains into the same protein (table S8.1).

Eumetazoan Networks and Pathways

How are the genes that were invented along the eumetazoan stem related to the organismal novelties associated with Eumetazoa? Satisfyingly, but perhaps not surprisingly, we found that the novel genes were significantly enriched for signal transduction, cell communication and adhesion, and developmental processes (*32*). The eumetazoan ancestor was the progenitor of all extant animals with nervous systems, and genes with neuronal activities are abundant among its novelties (Table 3). It is at first glance surprising that genes known to be involved in mesoderm development in bilaterians are also enriched among eumetazoan novelties, given that the textbook picture of cnidarians is that they lack mesoderm. Yet we know that many of these genes are associated either with basic patterning functions and/or the regulation of cell migration and fate. The precise deployment and interaction of these genes in the ancestral eumetazoan is therefore still a matter of debate (*26*, *27*, *59*–*61*). Experiments in cnidarians, however, in combination with information about mesodermal networks in bilaterians, could, in principle, constrain the ancestral genetic network and address whether or not the ancestor deployed these genes to generate this key germ layer.

Individual "new" genes are by themselves unlikely to bring about the suite of features needed to evolve animal characteristics from unicellular organisms. Rather, we expect that to generate organismal novelty, such new genes must be integrated with other novel and existing genes to evolve expanded or modified biochemical pathways and/or regulatory networks. Given the reconstructed eumetazoan genome and its various types of novel genes, we conclude by briefly considering selected eumetazoan pathways and processes to see how novel animal genes were incorporated into cellular and organismal functions.

Cell adhesion. In Bilateria, the integrin pathway mediates signaling from the extracellular matrix (ECM) that elicits various responses to modulate cell adhesion, motility, and the cell cycle (*62*). A detailed look at integrin signaling (Table 3 and Fig. 4B) reveals that most of the core components of the FAK and Fyn/Shc pathways were present in the eumetazoan ancestor. Various ancient cytosolic proteins (Talin, Paxillin, Grb2, Sos, and Crk) have been brought under the control of two novel receptors, integrin-α and integrin-β (the former being a type I novelty and the latter a type II novelty). FAK is a cytosolic component that appears as a type II novelty in eumetazoans, and calpain—a protease that regulates the aggregation of talin, paxillin, and FAK around the receptor—appears as a novel domain combination of ancient domains. Caveolin, a membrane adapter that couples the integrin-α subunit to Fyn is present in the *Nematostella* genome and is a type I novelty. Fyn itself is a more recent invention derived on the tetrapod stem by gene duplication.

Cell-cell adhesion mediated by cell-ECM interactions is a hallmark of animal multicellularity (*63*). Basement membrane proteins such as collagen and laminin arose as type II novelties along the stem leading to the Eumetazoa, whereas others such as nidogen are novel pairings of ancient domains (Table 1). Matrix metalloproteases also were invented as type II novelties, whereas guidance cues such as netrin and semaphorin that mediate adhesion are novelties with no evident homology to ancient eukaryotic proteins.

Signaling pathways. Animals rely on cell-cell signaling for cellular coordination during and after development (*64*). Various components of the Wnt and transforming growth factor–β (TGFβ) signaling pathways in the genome of *Nematostella* have been reported (*18*, *27*, *58*, *65*–*68*). In both pathways, the secreted ligands and their antagonists [such as Wnt, SFRP, bone morphogenetic

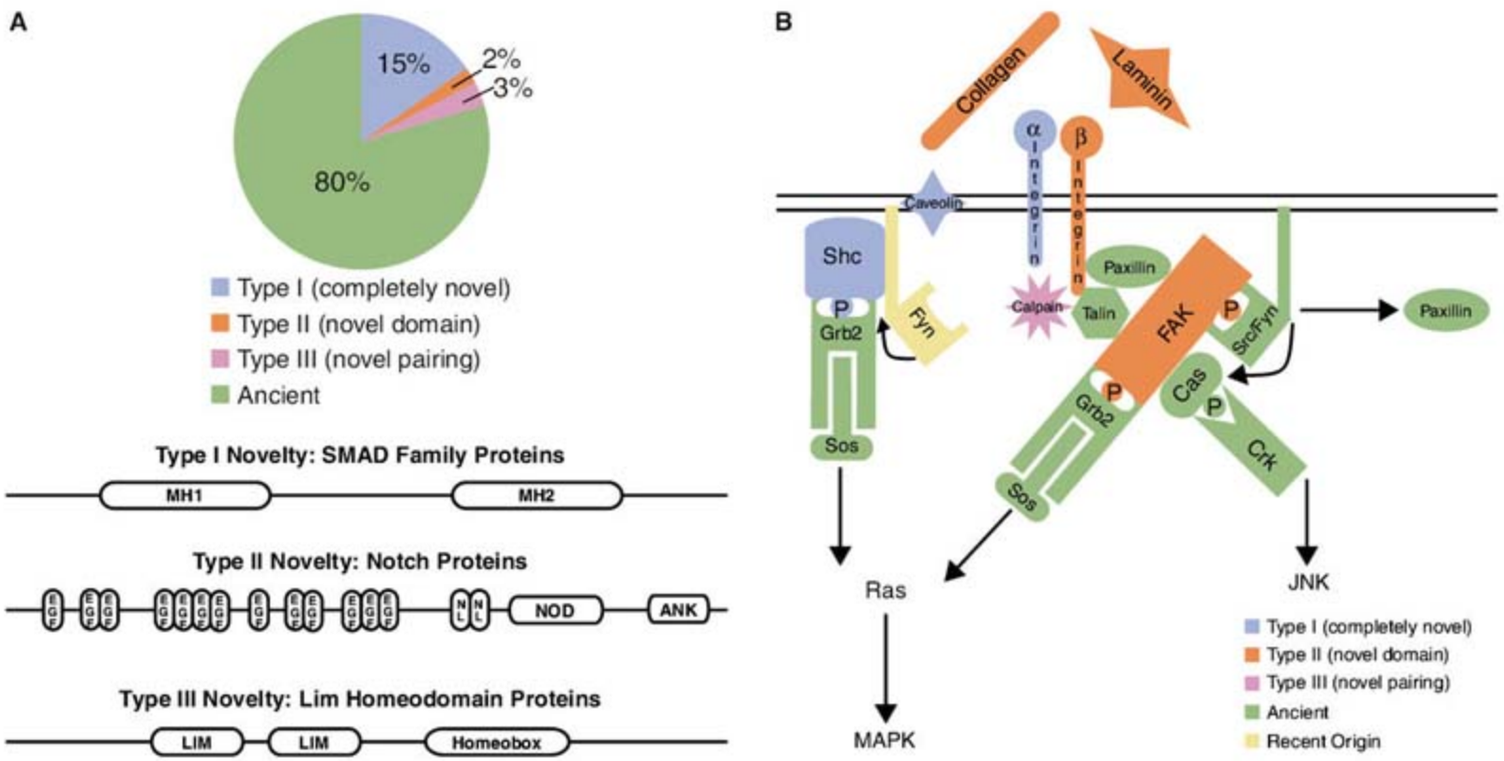


Fig. 4. Origins of eumetazoan genes. (**A**) Pie chart showing the percentages of genes in the eumetazoan ancestors according to their origin: type I novelties with no homology to proteins in nonanimal outgroups (blue), type II novelties with novel animal domains paired with ancient domains (orange), type III novelties with new pairings of ancient domains (pink), and ancient genes (green). (**B**) A schematic representation of the FAK and Shc/Fyn pathways in integrin signaling. The proteins are color-coded to reflect their ancestry, as in (A). JNK, c-Jun N-terminal kinase.

protein (BMP), and chordin] are novelties (Fig. 4B). Some, such as Wnt, secreted frizzled-related protein (SFRP), Dpp/BMP, activin, and chordin are type I novelties with no homology to proteins from outgroups; some are type II novelties (dickkopf), and others (such as tolloid) are novel pairings of ancient domains (type III). The receptor in the Wnt pathway, frizzled, also arose as a type I eumetazoan novelty. Transcription factors that are activated downstream of Wnt signaling are ancient, but the ones involved in TGFβ signaling are novel. Type I receptors of the TGFβ pathway arose as a pairing of novel animal domains with ancient domains (type II novelties) and type II receptors turn out to be ancient eukaryotic kinase genes that were co-opted for this function.

The presence of essentially complete signal transduction pathways in the common gene set of cnidarians and bilaterians suggests that the integration of novel eumetazoan genes into these systems was largely complete in the eumetazoan ancestor. A general trend in the evolution of signaling pathways may have been the co-option of cytosolic signaling components into pathways that could be regulated by newly invented ligands and receptors. For example, in the case of FGF signaling, the interactions of ancient cytosolic components [such as Grb2, Sos, and mitogen-activated protein kinase (MAPK)] could be elaborated with the addition of novel proteins (such as FGF and Shc) or of novel domains added to old proteins (such as Raf homolog) or novel pairings of old domains (such as FGF receptor and phospholipase C–γ).

Emergence of the neuromuscular system. Cnidarians and ctenophores are the earliest branching metazoan phyla that have a nervous system, although they lack overt centralization of the kind observed in bilaterians. Genes with neural functions in the Bilateria have been implicated in the cnidarian nervous system (*69*, *70*). Numerous genes known to be involved in neurogenesis, such as members of the homeobox and basic helix-loop-helix (bHLH) transcription factor families (Emx, Otp, Otx, and achaete-scute), can be traced to ancient eukaryotic genes with these signature domains. Some are novel pairings of ancient domains (such as neuropilin and LIM-homeobox genes), some are pairings of old domains with novel animal-specific domains (such as Dsh, Arx, and neuralized) and others are novel animal genes (such as Hes, Gcm, netrin, semaphorins, and dachsund). Certain enzymes important in synaptic transmission (such as 3,4-dihydroxy-L-phenylalanine (DOPA)–β monooxygenase) and some vesicular trafficking proteins (such as synaptophysin) appear as novel (type I) eumetazoan proteins. Regulatory subunits for ion channels important in nerve conduction and muscular function can be type I novelties (such as voltage-dependent calcium channel β subunit and potassium large-conductance calcium-activated channel) or type III novelties (such as voltage-dependent calcium channel α2/δ subunit). Various components of the dystrophin-associated protein complex (DPC) in the sarcolemma such as dystrophin, syntrophin, β-dystrobrevin, and β-sarcoglycan are type I novelties. Other sarcomere proteins are type II novelties (such as nebulin and tropomodulin). This diversity of origins of genes with roles in the neuromuscular system suggests that tracing the evolution of nerves and muscle will require

Table 2. Origins of developmental signaling pathway components inferred in the eumetazoan ancestor. ERK, extracellular signal–regulated kinase; MEK, MAPK kinase; GSK3, glycogen synthase kinase 3; APC, anaphase-promoting complex; TCF/LEF, T cell factor/lymphoid enhancer factor; ATF, activating transcription factor; ACVR2, activin receptor, type II; ADAM10, a disintegrin and metalloprotease domain 10; PEN2, presenilin enhancer 2; SYK, spleen tyrosine kinase; IGF, insulin-like growth factor; PTEN, phosphatase and tensin homolog; GTPase, guanosine triphosphatase; SOCS, suppressor of cytokine signaling; REL/NFκB, reticuloendotheliosis viral oncogene/nuclear factor κB; NFAT, nuclear factor of activated T cells; STAT5, signal transducers and activators of transcription 5.

Pathway	Type I novelty	Type II novelty	Type III novelty	Ancient gene
Integrin signaling	Integrin-alpha; caveolin	Collagen; Integrin-β; Fak; Jun	Calpain	Talin,; vinculin; paxillin; Ras; Grb2; SoS; Rap; ERK; MEK, Crk
Wnt signaling	Wnt; secreted frizzled related factors; frizzled; strabismus/van gogh	Dickkopf; arrow; dishevelled; axin		β-catenin; GSK3; APC; TCF/LEF; groucho
TGFβ signaling	Dpp/BMP; activin; gremlin; chordin; follistatin; R-SMAD; I-SMAD; co-SMAD	Type I receptors: TGFβR1, BMPR1A; ATF/JunB; snoN	Tolloid/BMP1	Type II receptors: ACVR2, BMPR2
Notch signaling	Numb; hairy/E(spl)	Notch		Jagged; deltex; fringe; presenilin; ADAM10; nicastrin; furin; Aph1; PEN2; mastermind
Ephrin signaling		Ephrin; Fak	Eph (receptor)	Abl/SYK
Insulin signaling	Insulin	Insulin receptor substrate; phosphoinositide-3-kinase, catalytic	Insulin receptor/IGF; phosphoinositide-3-kinase, class 2	phosphoinositide-3-kinase, class 3; phosphoinositide-3-kinase, regulatory subunit; 3-phosphoinositide-dependent protein kinase-1; PTEN
FGF signaling	FGF; Shc	Raf homolog serine/threonine-protein kinase; Ras GTPase activating protein	FGFR; RAS protein activator; phospholipase C–γ; phosphoinositide-3-kinase, class 2; protein kinase Cι	MAPK; phosphoinositide-3-kinase, class 3; Grb2; Protein kinase C; SoS; Rac
Cytokine signaling	Inositol 1,4,5-triphosphate receptor; SOCS; arrestin; guanine nucleotide binding protein γ; regulator of G-protein signaling; REL/NFKB; NFAT	Adenylate cyclase 5/6; STAT5; ATF/Jun	CDC42 binding protein kinase	MAPK; Rho kinase; Rho

detailed studies of the functions of these genes in organisms at the base of the metazoan tree.

Concluding Remarks

Modern animal genomes retain features inherited from the eumetazoan ancestor that have been elaborated on, and sometimes overwritten by, subsequent evolutionary elaborations and simplifications. Here, we compared the genomes of the sea anemone with diverse bilaterians, both to infer the content and organization of the genome of the eumetazoan ancestor and to trace the origins of uniquely animal features. In many ways, the ancestral genome was not so different from ours; it was intron-rich and contained nearly complete toolkits for animal biochemistry and development, which can now be recognized as pan-eumetazoan, as well as the core gene set required to execute sophisticated neural and muscular function. The ancestor had blocks of linked genes that remain together in the modern human and anemone genomes—the oldest known conserved synteny outside of prokaryotic operons. Whereas fruit flies and soil nematodes have proven to be exquisite model systems for dissecting the genetic underpinnings of metazoan development and physiology, their genomes are relatively poor models for the ancestral eumetazoan genome, having lost introns, genes, and gene linkages.

The eumetazoan ancestor possessed more than 1500 genes that are apparently novel relative to other eukaryotic kingdoms. Some are the result of domain shuffling, bringing together on the animal stem new combinations of domains that are shared with other eukaryotes. But many animal-specific genes contain sequences with no readily recognizable counterparts outside of animals; these may have arisen by sequence divergence from ancient eukaryotic genes, but the trail is obscured by deep time. Although we can crudely assign the origins of these genes to the eumetazoan stem, this remains somewhat unsatisfying. The forthcoming genomes of sponges, placozoans, and choanoflagellates will allow more precise dating of the origins and diversification of modern eumetazoan gene families, but this will not directly reveal the mechanisms for new gene creation. Presumably, many of these novelties will ultimately be traced back, through deep sequence or structural comparisons, to ancient genes that underwent extreme "tinkering" (*71*).

Table 3. Origins of selected metazoan processes inferred in the eumetazoan ancestor. CREB, cyclic adenosine 3′,5′-monophosphate response element–binding protein; HIF, hypoxia-inducible factor; CES, carboxylesterase; cGMP, guanosine 3′,5′-monophosphate; TNF, tumor necrosis factor; BOK, B cell leukemia/lymphoma 2–related ovarian killer; GULP, engulfment adaptor PTB domain containing; CRADD, caspase 2 and receptor-interacting serine-threonine kinase domain–containing adaptor with death domain; FMR, fragile X mental retardation syndrome; CARD, caspase recruitment domain family; SRGAP, Slit-Robo Rho GTPase activating protein; TNFRSF, TNF receptor superfamily; TRAF, TNF receptor–associated factor; SUMO, small ubiquitin-related modifier; L3MBT, Lethal(3)malignant brain tumor protein homolog; SKI, sarcoma viral oncogene homolog; AP-2, activating protein 2; MAF, musculoaponeurotic fibrosarcoma oncogene homolog; CBP, CREB-binding protein; ETO/MTG8, eighty twenty one/myeloid translocation gene 8.

Process	Type I novelty	Type II novelty	Type III novelty	Ancient gene
Neurogenesis	Hes; Gcm; Ephrin; netrin; semaphoring; dachsund; ski oncogene	Notch; NGFR; Dsh; Arx; CREB/ATF; neuralized	Neuropilin; Lhx; ephrin receptor	Single-minded/HIF; achaete-scute; elav; Emx; Otp; Jagged; Deltex; Irx; Gli, Otx/Phox; stonal/neuroD/neuroG; reticulon
Synaptic transmission	Nitric oxide synthase (neuronal) adapter protein; DOPA-β monoxygenase; calcium channel voltage-dependent β; syntrophin; synaptophysin; dystrophin; potassium large conductance calcium-activated channel, subfamily M β	Cholinergic receptor, nicotinic; neurexin	K-voltage gated channel; discs large	Glutamate receptor; synaptotagmin; intersectin; synapsin; neuroligin/CES; syntaxin; glutamate transporter
Extracellular matrix	Netrin; dermatopontin; semaphorin; glypican; stereocilin	Collagen; spondin; laminin	Nidogen; stabilin; neuropilin; matrix metalloprotease; thrombospondin	Leprecan; microfibrillar-associated protein
Cell junction	par-6	Tight junction protein		Salvador
Muscle contraction	Voltage-dependent calcium channel β, β-sarcoglycan, β-dystrobrevin	Cholinergic receptor, nicotinic; nebulin; tropomyosin; calponin/transgelin	Voltage-dependent calcium channel α2/δ subunit; inositol triphophate receptor; calcium activated potassium channel slowpoke	Phosphorylase kinase; myosin light chain cytoplasmic; calcium channel alpha subunit; cGMP-dependent protein kinase; calcium/calmodulin-dependent kinase II; myosin regulatory light chain
Apoptosis	TNF5/10/11; Bcl2; BOK; GULP; CRADD; caspase 8/10; growth arrest and DNA-damage-inducible; DNA fragmentation factor 40-kD subunit; interleukin enhancer-binding factor 3; FMR	Neuronal apoptosis inhibitory protein; CARD9/11	NGFR; SRGAP; calpain	TNFRSF; TRAF; scavenger receptor class B; huntingtin interacting protein; programmed cell death 1/5; Bcl2-associated athanogene; Akt; SUMO; defender against cell death 1; apoptosis-inducing factor–like mitochondrion-associated inducer of death; death-associated protein kinase
Transcription factors	L3MBT; T-Box; Nuclear hormone receptor; SMAD; dachsund; gcm; NFAT; nuclear respiratory factor; SKI family; sprouty; AP-2; onecut; MAF-related	CBP/p300; ETO/MTG8/Nervy; groucho; Jun; Myt1; runt; STAT	Hairless; nuclear protein 95; LIM homeobox; CCAAT enhancer binding; aryl hydrocarbon receptor related	Zic; Gli; homeobox; bHLH; achaete-scute; sox; retinoblastoma binding protein 5/8; NFKB-related; Krueppel C2H2 type zinc finger; Irx; Deltex; ataxin

The eumetazoan progenitor was more than just a collection of genes. How did these genes function together within the ancestor? Unfortunately, we cannot read from the genome the nature of its gene- and protein-regulatory interactions and networks. This is particularly vexing as it is becoming clear—especially given the apparent universality of the eumetazoan toolkit—that gene regulatory changes can also play a central role in generating novelties, allowing co-option of ancestral genes and networks to new functions (*49*). Of particular interest are the processes that give rise to body axes, germ layers, and differentiated cell types such as nerve and muscle, as well as the mechanisms that maintain these cells and their interactions through the growth and repair of the organism. *Nematostella* and its genome provide a platform for testing hypotheses about the nature of ancestral eumetazoan pathways and interactions, with the use of the basic principle of evolutionary developmental biology: Processes that are conserved between living species were likely functional in their common ancestor.

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Supporting Online Material

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Materials and Methods
SOM Text
Figs. S1.1 to S7.4
Tables S1.1 to S8.1
References

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Dentate Gyrus NMDA Receptors Mediate Rapid Pattern Separation in the Hippocampal Network

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Forming distinct representations of multiple contexts, places, and episodes is a crucial function of the hippocampus. The dentate gyrus subregion has been suggested to fulfill this role. We have tested this hypothesis by generating and analyzing a mouse strain that lacks the gene encoding the essential subunit of the *N*-methyl-D-aspartate (NMDA) receptor NR1, specifically in dentate gyrus granule cells. The mutant mice performed normally in contextual fear conditioning, but were impaired in the ability to distinguish two similar contexts. A significant reduction in the context-specific modulation of firing rate was observed in the CA3 pyramidal cells when the mutant mice were transferred from one context to another. These results provide evidence that NMDA receptors in the granule cells of the dentate gyrus play a crucial role in the process of pattern separation.

The hippocampus is crucial for the formation of memories of facts and episodes (*1*–*4*). To allow similar episodes to be distinguished, it must rapidly form distinct representations of the temporal and spatial relationships comprising events (pattern separation), and because specific episodes are rarely replicated in full, the hippocampus must also be capable of

using partial cues to retrieve previously stored representations (pattern completion). Specific hippocampal subregions and circuits have been suggested to subserve these mnemonic requirements: the feedforward pathway from the entorhinal cortex (EC) to the dentate gyrus (DG) and on to CA3 for pattern separation, and the recurrent and highly plastic connections in CA3 for pattern completion (*5*–*8*). Recently, targeted genetic manipulations provided strong evidence for the role of plastic CA3 recurrent synapses in pattern completion (*9*, *10*). However, evidence supporting the hypothesis of pattern separation at the behavioral level has been scant and limited to interpretation of impairments observed in rodents with DG lesions (*11*, *12*)

The activity of hippocampal neurons ("place cells") depends on an animal's location in the environment (*13*), and many studies suggest that ensemble place cell activity encodes memory traces (*14*–*18*). Recent studies have directly investigated whether physiological correlates of behavioral pattern separation can be detected in the hippocampal circuits by means of the place cell recording technique (*19*–*26*). One finding particularly relevant to our current study (*20*) is that exposure of rats to two similar but distinct contexts generates place cell firing rates in the two contexts that overlap significantly less in CA3 than in CA1. Although consistent with the hypothesis (*6*–*8*) that the DG helps separate the overlapping representation patterns as they reach the CA3 region, this study did not directly test the role of the EC→DG→CA3 circuit in behavioral and physiological separation, nor propose a mechanism for the process.

Generating dentate gyrus granule cell–specific NMDA receptor knockout mice. In one of several mouse lines using a proopiomelanocortin (POMC)–bacterial artificial chromosome to drive expression of the Cre recombinase (*27*), crossing with lacZ reporter mice [Rosa26 (*28*)] revealed robust Cre-loxP recombination in the DG granule cell (GC) layer throughout the dorsal/ventral axis (Fig. 1, A to D), with sparser recombination in the arcuate nucleus of the hypothalamus, the lateral habenular nucleus, and a small number of scattered cortical and midbrain cells. Immunofluorescence studies with antibodies specific for β-galactosidase (a Cre-loxP recombination marker), NeuN (a neuronal marker), S100β (a glial cell marker), and glutamic acid decarboxylase (GAD-67, an interneuron marker) indicated that the Cre-loxP recombination is confined to GCs in the DG of the hippocampus (Fig. 1, E to K). Cre-loxP recombination in the DG GC layer begins between postnatal weeks 2 to 3 and remains spatially restricted until at least 24 weeks of age (fig. S1). It is known that DG GCs can arise via adult neurogenesis. Cre-loxP recombination is detected in newly born neurons that had reached the GC layer (Fig. 1, L to N).

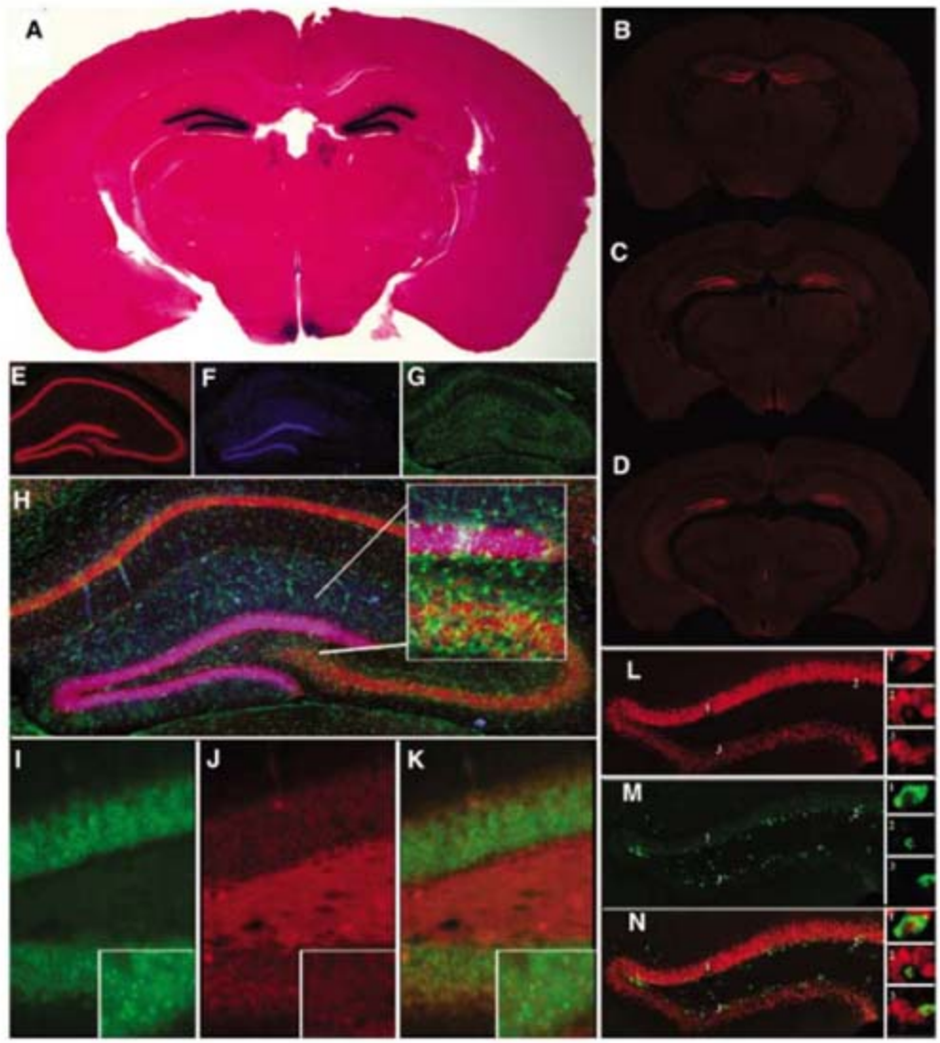

Fig. 1. Basic features of POMC-Cre transgenic mice. (**A**) Image (1× magnification) of β-galactosidase (β-Gal) expression in a 12-week-old POMC-Cre/ROSA26 double-transgenic mouse stained with X-Gal and nuclear fast red. (**B** to **D**) Anti–β-Gal immunohistochemical (1×, images visualized with Cy3, red) staining showing expression in a 16-week-old POMC-Cre/ROSA26 double-transgenic mouse in three coronal sections taken along the rostro-caudal axis of the forebrain. (**E** to **K**) Immunofluorescence staining of coronal sections of a 20-week-old POMC-Cre/ROSA26 double-transgenic mouse. Single staining with (E) anti-NeuN (neuronal marker; AlexaFlour 555, red, 4×), (F) anti–β-Gal (marker of Cre recombination; aminomethylcoumarin, blue, 4×), and (G) anti-s100β (glial cell marker; fluorescein isothiocyanate, green, 4×). (H) A 4× merge of (E), (F), and (G) indicating that the Cre-loxP recombination is restricted to the neurons in the DG. Inset is a 20× image of the DG. (I to K) Single staining with (I) an anti–β-Gal (AlexaFlour488, green, 20×) and (J) anti-GAD67 (marker of inhibitory neurons; Cy3, red, 20×). (K) A merge of (I) and (J), indicating no recombination in GAD-67–positive inhibitory neurons. In the bottom right corner of each figure is a magnified image of several cells in the upper blade of the DG showing the separation of the green and red signals. (**L** to **N**) After injection of bromodeoxyuridine (BrdU) into a 12-week-old male POMC-Cre/ROSA26 double-transgenic mouse, brain sections were imaged by confocal microscopy. To the right of each 10× image are three single cells imaged at 63× at the positions in the 10× image labeled with the corresponding number. Single staining with (L) anti–β-Gal (Cy3, red) and (M) anti-BrdU (marker of newly born cells; AlexaFlour488, green). (N) The overlap of the green and red signal in the cells imaged indicates that recombination occurs in newly born neurons after they reach the GC layer.

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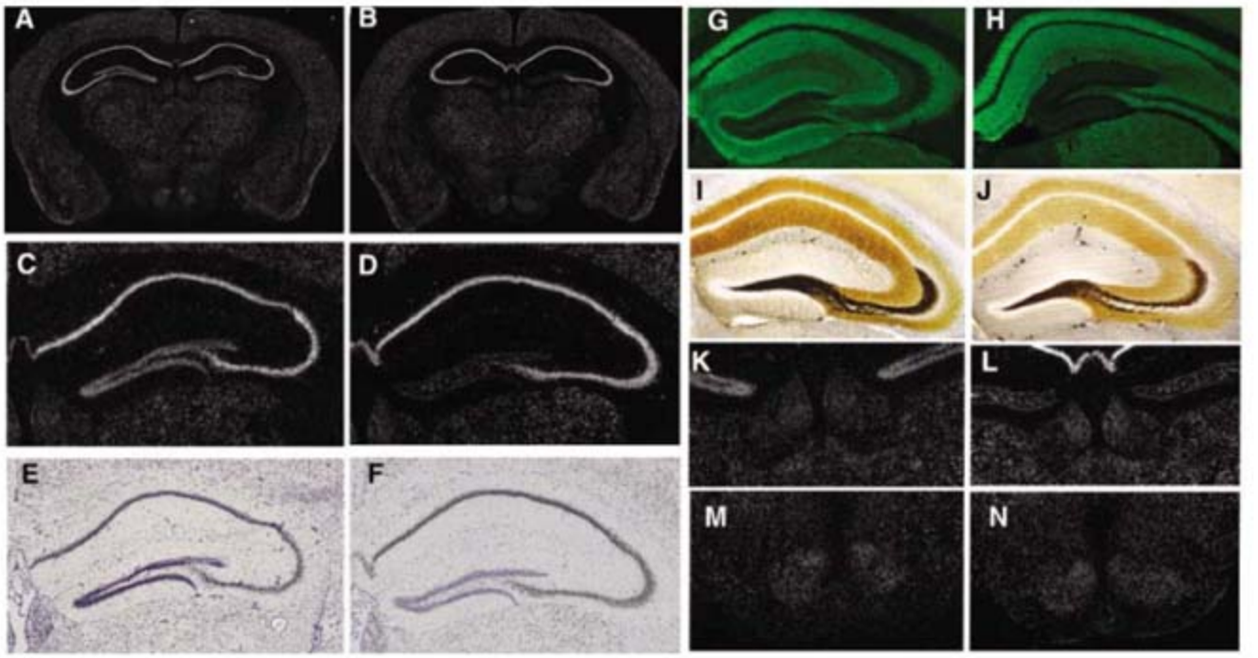

Fig. 2. Basic features of DG-NR1 KO mice. (**A** to **F**) Images of coronal sections after in situ hybridization with a ^{33}P-labeled NR1 cDNA probe. (A) Dark-field image (1× magnification) of a midbrain coronal section from a 20-week-old *f*NR1 control male; (B) 1× image of a midbrain coronal section from a 20-week-old DG-NR1 KO male. (C) Image (4× magnification) of the hippocampus from the control mouse; (D) 4× image of the mutant mouse hippocampus. The NR1 transcript is deleted specifically in the DG granule cell layer in the mutant mouse. Light-field image of (E) the *f*NR1 hippocampus and (F) the DG-NR1 KO hippocampus reveal no changes in the gross structure of the hippocampus. (**G** and **H**) Immunohistochemical labeling of the NR1 protein (visualized with AlexaFlour 488, 4×) in the hippocampus of (G) a *f*NR1 animal at 16-weeks of age and (H) a DG-NR1 KO littermate. There is complete and specific loss of the receptor in the dentate gyrus of the KO mouse. (**I** and **J**) Timms staining of the mossy fiber pathway in (I) a 20-week-old *f*NR1 mouse and (J) a mutant littermate revealed no changes in the structure of the DG outputs as a result of the mutation. (**K** to **N**) In situ hybridization with the NR1 probe did not indicate a reduced NR1 mRNA level elsewhere in the brains of the knockout mice. Examination of 4× dark-field images of (K) the habenular nucleus of a 20-week-old *f*NR1 control animal and (L) a DG-NR1 KO littermate, and (M) the arcuate nucleus of control and (N) DG-NR1 KO mouse, found no difference in the abundance of the transcript in either region in the mutant mice.

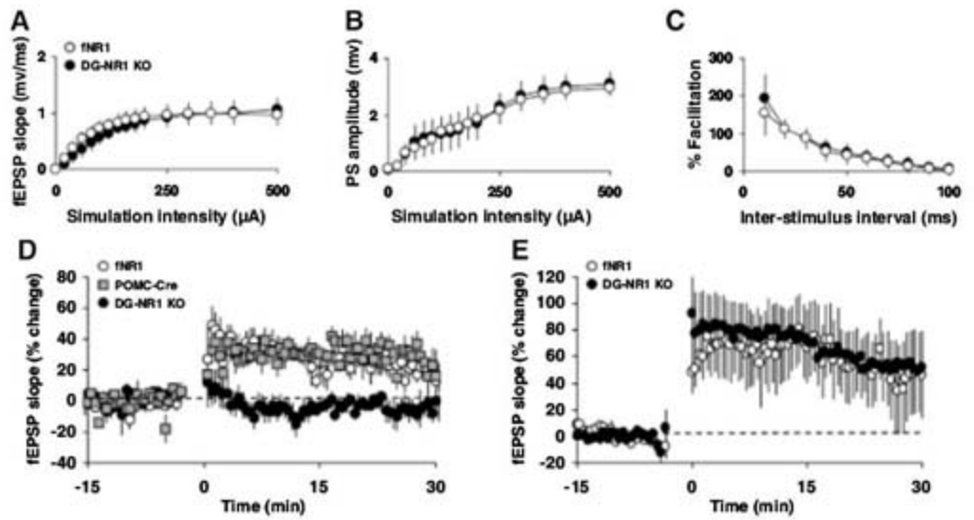

Fig. 3. In vivo synaptic transmission and plasticity of the DG-NR1 KO mice. PP-GC input/output curves of *f*NR1 control (open circles) and mutant (filled circles) mice, showing similar fEPSP slopes (**A**) and population spike (PS) amplitudes (**B**) at all stimulation intensities. (**C**) Paired-pulse facilitation at PP-GC synapses also appeared normal in the mutant mice. (**D**) Theta-burst stimulation of the PP input to the DG induced potentiation of fEPSP in *f*NR1 (open circles) and POMC-cre control animals (squares), but not in DG-NR1 KO mice (filled circles). In contrast, high-frequency stimulation of Schaffer commissural input induced (**E**) CA1 LTP in both DG-NR1 KO mice and *f*NR1 controls. Error bars are the SEMs.

We generated DG-NR1 knockout (KO) mice by crossing these POMC-Cre mice with floxed NR1 (*f*NR1) mice (*29*). In situ hybridization showed that NR1 RNA begins to decrease sometime between 1.5 and 4 weeks after birth and is nearly absent by 16 weeks of age in the DG GCs (Fig. 2, A to F, and fig. S1). Immunocytochemistry with antibodies to NR1 (anti-NR1) showed a total absence of DG GC NR1 protein by 16 weeks of age (Fig. 2, G and H), whereas the hippocampal cytoarchitecture appeared normal (Fig. 2, I and J). We were unable to detect any reduction in the levels of NR1 mRNA or protein in CA1 or CA3 pyramidal cells (Fig. 2, C to H, and fig. S1), the arcuate nucleus of the hypothalamus, neocortex, or the habenular nucleus (Fig. 2, K to N). Furthermore, we did not detect any changes in activity, feeding, reproductive, or parental behaviors in our DG-NR1 KO mice, or in body weight under free-feeding or food-restricted conditions (fig. S2).

Perforant path–dentate gyrus synaptic transmission and plasticity. We examined in vivo synaptic transmission and plasticity in the hippocampus of anesthetized DG-NR1 KO mice and control littermates of 20 to 24 weeks of age. When we stimulated the perforant path (PP) and recorded population responses in the dentate hilus, we found no differences between input-output curves in DG-NR1 KO mice ($n = 5$) and *f*NR1 littermate controls ($n = 4$) (Fig. 3, A and B). Paired-pulse facilitation—an index of presynaptic function—also appeared normal in the mutant mice (Fig. 3C). Theta-burst stimulation of the PP evoked robust potentiation of the PP-GC synapses as measured by an increase in the slope of the field excitatory postsynaptic potential (fEPSP) in both the POMC-Cre control mice ($n = 3$; 30 ± 8.1%) and the *f*NR1 control mice ($n = 4$; 38 ± 7.7%, $P = 0.008$) (Fig. 3D). In contrast, identical protocols failed to evoke plasticity in DG-NR1 KO mice ($n = 5$; change in fEPSP slope = −2.6 ± 6.6%, $P = 0.88$; DG-NR1 KO × *f*NR1 littermates, $P = 0.004$) (Fig. 3D). Long-term potentiation (LTP) in area CA1 after high-frequency stimulation of the CA3 Schaffer commissural inputs was normal (*f*NR1: 66 ±

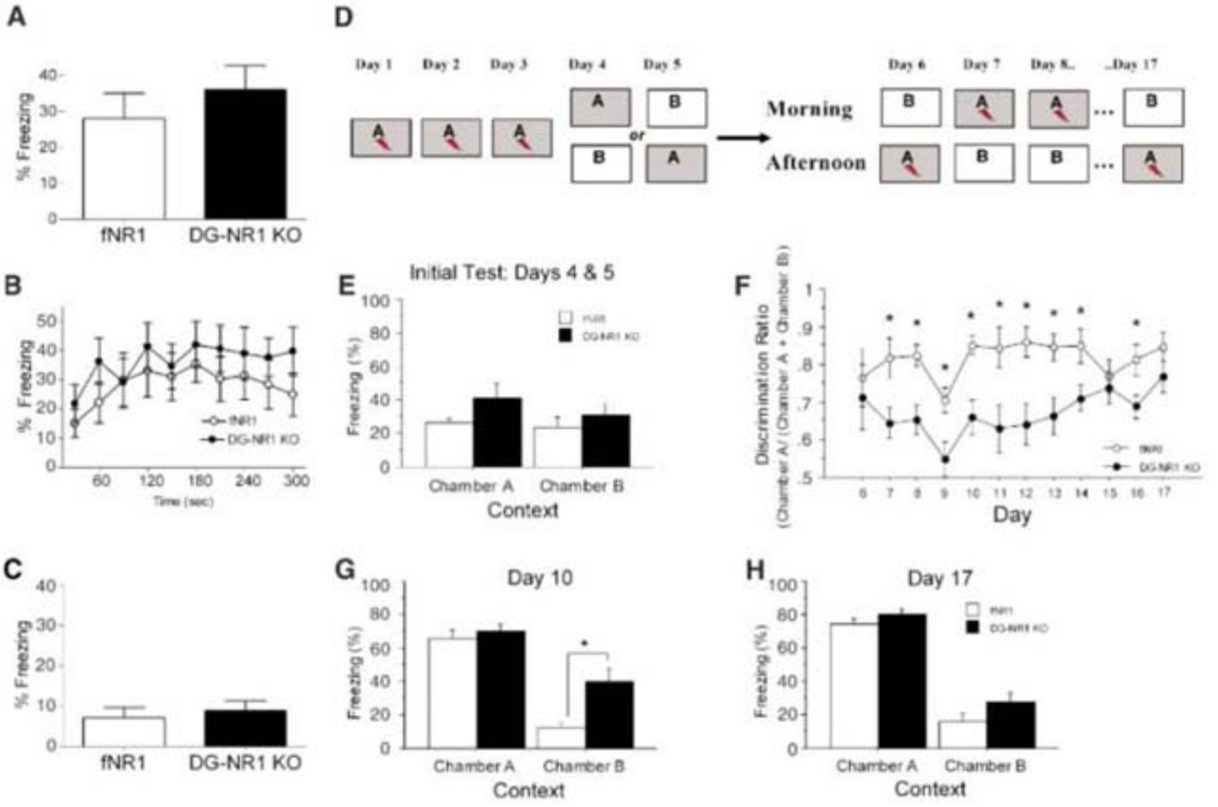

Fig. 4. DG NRs are important for the discrimination of similar contexts. (**A** and **B**) Contextual fear was measured 48 hours after conditioning with a single 2-s 0.75-mA footshock. Both *f*NR1 mice (n = 12) and DG-NR1 KO littermates (n = 12) showed (A) elevated total freezing in the conditioned context, as well as (B) identical kinetics of freezing across the 5-min test. (**C**) Generalized freezing behavior to a second, very different context was low in both genotypes. Separate groups of mice were subjected to a protocol (**D**) designed to test contextual discrimination. For the first 3 days of conditioning, mice visited only chamber A and each day received a single footshock (2 s, 0.65 mA). Freezing was measured once in chamber A and once in chamber B over the subsequent 2 days, and (**E**) control and mutant mice displayed equal amounts of freezing in both chambers. During days 6 to 17, mice visited each chamber daily (receiving a shock in one of the two), and freezing was assessed during the first 3 min in each chamber. (**F**) *f*NR1 mice (open circles; n = 12) showed significantly greater discrimination than the DG-NR1 KO mice (filled circles; n = 12) across most of the acquisition. (**G** and **H**) Freezing in chamber A and chamber B for the control (open bars) and mutant (filled bars) on (G) day 10 (middle of discrimination) and (H) day 17 (end of discrimination) demonstrated that the initial DG-NR1 KO discrimination deficit was rescued with additional training. Error bars in (B) to (H) are the SEMs.

Table 1. Basic properties of pyramidal cells recorded in CA1 and CA3 during exploration of the white circular arena (run 1) on the first day of the pattern separation experiment. Values are means ± SEM. *N*, number of mice; *n*, number of cells.

Measurement	CA1		CA3	
	*f*NR1 (N = 5, n = 32)	DG-NR1 KO (N = 5, n = 46)	*f*NR1 (N = 5, n = 26)	DG-NR1 KO (N = 6, n = 26)
Mean firing rate (Hz)	0.384 ± 0.047	0.728 ± 0.149	0.378 ± 0.069	0.322 ± 0.049
Spike width (μS)	598 ± 7.65	560 ± 24.8	554.2 ± 12.8	530.5 ± 31.9
Complex spike index (bursting)	18.26 ± 1.76	20.37 ± 1.62	20.29 ± 2.20	22.05 ± 1.96
Peak rate (Hz)	20.53 ± 1.25	18.08 ± 1.37	12.58 ± 1.85	13.07 ± 1.20
Field size (% of sampled space)	24.68 ± 1.78	31.8 ± 2.63*	28.14 ± 3.02	35.9 ± 3.19

*Significantly different from *f*NR1 control (Student's t test, $P < 0.05$).

25%, P = 0.04; DG-NR1 KO: 88 ± 21%, P = 0.04) (Fig. 3E).

Hippocampal memory and discrimination. We first subjected the DG-NR1 KO and control *f*NR1 littermates to the hidden platform version of the Morris water maze and found no detectable deficit in the mutants with the standard protocol (fig. S3). DG-NR1 KO mice also acquired and retained contextual fear conditioning as efficiently as control littermates after a single context-footshock pairing [mice aged 16 to 24 weeks; *f*NR1 control: 28.1 ± 6.9%; DG-NR1 KO: 36.1 ± 6.6%, P = 0.41; two-way analysis of variance (ANOVA): genotype × minute $F(1,9) = 0.65$, P = 0.52; genotype $F(1,9) = 0.70$, P = 0.41; minute $F(1,9) = 4.0$, $P < 0.001$) (Fig. 4, A and B). We examined the context specificity of the conditioning by assessing "freezing" behavior in a second context in which the chamber floor was changed from a metal grid to smooth plastic and the ambient lighting was changed from white to red (*30*). This distinct context evoked significantly lower levels of freezing (similar in the two genotypes) than the conditioned context (Fig. 4C) (*f*NR1 control: 7.1 ± 2.6%; DG-NR1 KO: 8.9 ± 2.4%; ANOVA, genotype × chamber $F(1,1) = 0.37$, P = 0.55; genotype $F(1,1) = 0.92$, P = 0.34; chamber $F(1,1) = 22.28$, $P < 0.0001$; *f*NR1 conditioned × nonconditioned, $P < 0.05$; DG-NR1 KO conditioned × nonconditioned, $P < 0.001$).

To investigate whether the NRs in the DG GCs play a role in behavioral pattern separation, we subjected the DG-NR1 KO mice to contextual fear conditioning using a less distinct pair of contexts (A and B) that shared an identical metal grid floor (*30*), but had unique odors, roofs, and lighting (*31*). In this protocol, conditioning took place incrementally over several days, allowing the effects of repeated experiences to be investigated (*32*) (Fig. 4D). On the first 3 days of the experiment, the mice were placed only into chamber A where, 192 s after being placed, they received a single footshock. On day 4, the mice of each genotype were divided into two groups, with one group of each genotype visiting chamber A and the other visiting chamber B; no group received a footshock in either chamber, and freezing was assessed. On day 5, each mouse visited the chamber opposite to the one visited on day 4, and freezing in the absence of footshock was assessed again. After this contextual conditioning there were no freezing differences between genotypes in chamber A, confirming the ability of the mutant mice to acquire contextual fear conditioning. However, because of a greater similarity between chamber A and chamber B compared to those used in the experiment of Fig. 2, A to C, there was extensive generalization between contexts in both genotypes [$F(1,20)$ =

0.66, $P = 0.43$] (Fig. 4E). During the subsequent discrimination phase of the task, mice visited the two chambers daily for 12 days (day 6 to day 17), always receiving a footshock 192 s after being placed in chamber A, but never in chamber B. Freezing during the first 3 min in each chamber was used to calculate a daily discrimination ratio (Fig. 4F). Control mice quickly learned to distinguish the chambers; however, the DG-NR1 KO mice exhibited a transient, yet very significant, deficit during the acquisition of the discrimination task [$F(1,20) = 15.11$, $P < 0.001$) (Fig. 4F). This deficit in the mutant mice was exhibited as elevated freezing in the shock-free chamber B [two-way ANOVA, chamber × genotype interaction $F(1,20) = 8.70$, $P < 0.008$; pairwise comparison of chamber B freezing, $P < 0.05$] (Fig. 4G). In contrast, at no point during the task did the mutant animals demonstrate a deficit in freezing to the context paired with shock (chamber A). By day 17 (the 12th day of the discrimination task), the DG-NR1 KO mice could discriminate the two chambers in a manner indistinguishable from that of control mice [two-way ANOVA, chamber × genotype interaction $F(1,20) = 0.84$, $P = 0.37$] (Fig. 4H).

Hippocampal place cells and contextual discrimination. We used multi-tetrode recordings to monitor hippocampal ensemble activity as DG-NR1 KO mice explored two distinct contexts. Mice foraged for scattered food rewards in an open, white, circular low-walled box for a 10-min habituation session. Twenty-four hours later, CA1 and/or CA3 place cell activities were recorded in the same box for 10 min (run1). Mice were then placed in a small "sleep" box for 20 min while the white circular box was replaced with an open, black, square low-walled box, and then animals returned to this new box for a second 10-min run session (run2). Forty-six CA1 pyramidal cells and 26 CA3 pyramidal cells from six mutant mice (age 16 to 24 weeks) and 32 CA1 pyramidal cells and 26 CA3 pyramidal cells from five *f*NR1 control mice (littermate controls; age 16 to 24 weeks) met our threshold of a 0.2-Hz average firing rate in at least one of the two boxes (*20–22*, *31*) (fig. S4 and table S3). Run1 spike widths, complex spike indices, peak firing rates, and average firing rates were similar across genotypes in both CA1 and CA3 (Table 1). However, DG-NR1 KO mice displayed larger place fields in CA1 ($P < 0.05$). Figure 5A shows example rate maps of 10 CA3 neurons from each of the two genotypes in both recording contexts (see also table S2).

We assayed rate remapping in both CA1 and CA3 by comparing the average firing rates of each unit during exploration of the two boxes using (i) the normalized change in average firing rate between the two boxes (rate difference) and (ii) a ratio of firing rates in the two contexts (rate overlap) (*20–22*). In control mice, the rate difference in the CA3 region (0.352 ± 0.043) was significantly greater than that in the CA1 region (0.238 ± 0.037; Bonferroni post-test, $P < 0.01$). In contrast, DG-NR1 KO mice showed similar rate differences in CA3 and CA1 (0.184 ±.0.022 versus 0.199 ± 0.037, respectively; $P = 0.97$) (table S1 and fig. S6). The mutation had a significant effect on the rate difference, with the values measured in the CA3 of the mutant mice significantly lower than those in the CA3 of the control mice [two-way ANOVA, genotype × region $F(1,1) = 3.52$, $P = 0.06$; genotype $F(1,1) = 9.18$, $P = 0.003$; region $F(1,1) = 2.09$, $P = 0.15$; Bonferroni post-test, *f*NR1 CA1 × DG-NR1 KO CA1, $P > 0.05$; *f*NR1 CA3 × DG-NR1 KO CA3, $P < 0.01$) (Fig. 5B). To assess if these rate differences reflected significant rate remapping, we compared actual rate difference values with rate differences for each region expected if the firing rates in the two boxes were independent of one another (Fig. 5B, red lines). Rate differences were significantly lower than expected under fully independent conditions in both CA1 and CA3 of the mutants (CA1 $Z = 5.44$, $P < 0.001$; CA3 $Z = 3.12$, $P < 0.001$), as well as in CA1 of the controls (CA1 $Z = 2.99$, $P < 0.002$). Only the control CA3 data showed evidence of independent firing rates in the two boxes (CA3 $Z = 0.44$, $P = 0.33$). Figure 5C shows the cumulative probability histogram for the rate overlap in CA3 and CA1 regions. In CA3 of control mice, there was a significant shift of the histogram to the left compared to the CA1 (Mann Whitney *U* test; $P < 0.04$), indicating a greater proportion of CA3 cells undergoing rate remapping. In the KO mice, the distributions of rate overlap values in both CA1 and the CA3 were significantly shifted to the right of the control CA3 histogram and were similar to that of the control CA1 histogram (control CA3 × mutant CA3, $P < 0.01$; control CA3 × mutant CA1, $P < 0.003$).

Place cells can show context-dependent changes in firing location as well as firing rate ["global remapping" (*20*)]. We therefore compared the locations of CA1 and CA3 place fields

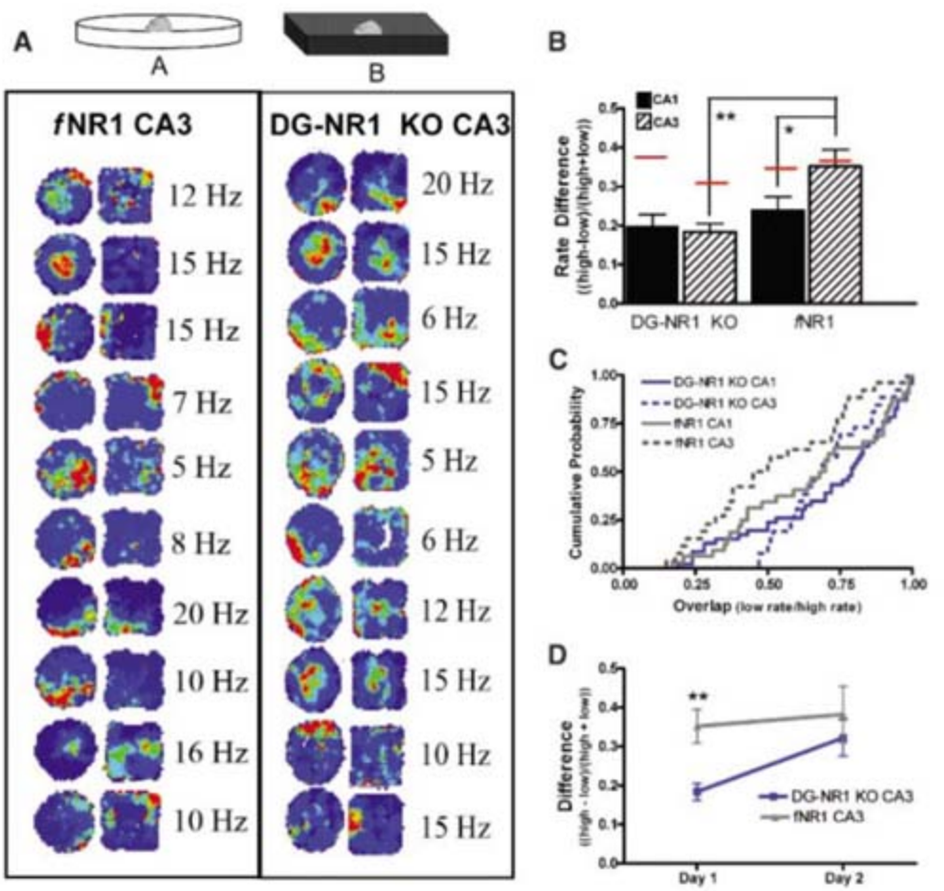

Fig. 5. DG NRs are important for context-specific modulation of firing rate in CA3. (**A**) Examples of firing-rate maps showing the activity of 10 *f*NR1 control (left) and 10 DG-NR1 KO (right) CA3 place cells as mice successively explored a white circular box and a black square box in the same location. Colors are scaled to maximum firing rates given by the numbers (red, maximum; blue, silent). (**B**) The rate difference in average firing rate in the two boxes was calculated for each cell [(high rate − low rate)/(high rate + low rate)]. *f*NR1 control rate differences were larger for CA3 than for CA1, whereas DG-NR1 KO rate differences were similar in CA3 and CA1. Mutant CA3 rate differences were significantly smaller than those of control mice (*$P < 0.05$, **$P < 0.01$). Red lines indicate rate differences expected given independent firing in the two boxes. (**C**) Cumulative probability histograms of the overlap [(low rate)/(high rate)] values for both genotypes and subregions showing significantly greater rate remapping (leftward shift) in control mice. (**D**) When the two box/one room experiment was repeated 24 hours later, DG-NR1 KO also showed significant rate remapping in CA3 (**day 1 $P < 0.01$; day 2 $P = 0.57$). Error bars in (B) and (D) are the SEMs.

in the two contexts by binning averaged firing rates into positional pixels and measuring the distance between peak firing rate pixels in the black and white boxes. Positional remapping was similar across subregions, conditions, and genotypes (mean intercontext shift of 3.0 ± 0.3 and 3.7 ± 0.4 pixels in control CA1 and CA3; 2.7 ± 0.3 and 3.4 ± 0.4 in DG-NR1 KO CA1 and CA3) (SOM Text and fig. S5). Thus, the remapping deficits in DG-NR1 KO mice were confined to the rate remapping dimension under these conditions.

The deficit revealed by the contextual fear discrimination paradigm was limited to the early days of training (Fig. 4, F and G). We therefore investigated whether the deficit in context-mediated firing-rate modulation in the CA3 region of the mutant mice could be overcome with more experience. When the mice were allowed to return to the recording room 24 hours later and the recording protocol was repeated, we found similar rate differences in the CA3 regions of both genotypes (15 CA3 cells in five *f*NR1mice, 0.382 ± 0.072; 20 CA3 cells in four DG-NR1 KO mice, 0.322 ± 0.047; P=0.58). Although we could not detect a significant genotype × day interaction, post-tests revealed that the rate remapping deficit was not present on day 2 in the mutant mice [two-way ANOVA, genotype × day $F(1,1) = 1.45$, $P = 0.23$; genotype $F(1,1) = 6.53$, $P = 0.01$; day $F(1,1) = 3.54$, $P = 0.06$; Bonferroni post-test day1 *f*NR1 × DG-NR KO, $P < 0.01$; day 2 *f*NR1 × DG-NR KO, $P > 0.05$] (Fig. 5D).

Discussion. Using conditional genetic-engineering techniques, we have previously shown that NRs in the CA3 play a crucial role in rapid learning and pattern completion–mediated recall, whereas CA1 NRs are required for the formation of both spatial and nonspatial memory (*9*, *10*, *29*, *33*–*35*). The DG-NR1 KO mice described here allowed us to extend the study to the roles of DG NRs and NR-dependent plasticity. Our data support the notion that DG GC NRs play an important role in rapidly forming a unique memory of a context and discriminating it from similar contexts previously encountered (pattern separation), although they are dispensable for the acquisition of contextual memory per se.

DG-NR1 KO mice exhibited impaired context discrimination early during training in the incremental fear-conditioning paradigm and impaired context-modulated place cell activity in CA3 on the initial day of recording. Both deficits were overcome with training or experience. Together, these data suggest that NR function at PP-GC synapses is important for the animals' ability to discriminate similar contexts rapidly with limited experience, but not for slower acquisition of this ability over more trials. We suggest that common mechanisms underlie the DG-NR1 KO deficits observed at both the behavioral and physiological levels, despite the different timelines of recovery to control levels. These differences may reflect differences between the cues used to define the contexts, the use of conditioning footshocks in the behavioral experiment (*36*), the contribution of non-hippocampal structures in fear conditioning, or the greater sensitivity of the readout in the place cell recordings relative to the behavioral task. The eventual acquisition of the discriminating power by DG-NR1 KO mice may be due to the gradual development of synaptic plasticity at sites downstream of the PP-GC synapses. For example, the recurrent collateral-CA3 synapses may provide a complementary site at which small differences in PP input can be amplified; this is supported by a recent study reporting a contribution of CA3 NRs to pattern separation (*37*). Although the large number of cells and sparse connectivity of the DG would provide the ultimate substrate for the pattern separation, synaptic plasticity may be the tool that allows rapid and efficient separation of representations.

CA3 receives excitatory input from two external sources, the DG and the EC. Input from the DG is most likely to contribute to the orthogonalization of CA3 representations by virtue of the high GC number and the sparse GC-CA3 connectivity. Loss of NRs in the GCs may decrease drive from the DG to CA3. This would increase the relative proportion of EC drive to CA3, thus reducing the CA3 ensemble's ability to detect, amplify, and reflect small differences in EC activity generated in similar contexts. Indeed, rate remapping in CA3 (induced by changes in recording chamber shape or color) can occur in the absence of detectable changes in medial EC firing rates or locations (*25*, *26*). However, despite unvarying input from the EC, DG GCs did respond to contextual changes robustly and rapidly under these conditions. Our data suggest that NR-mediated activity or plasticity in the GCs may underlie these changes, subsequently shaping CA3 encoding.

It is puzzling that rate remapping in CA3 did not always affect spatial or rate coding downstream in CA1 (Fig. 5B) (*20*). It is possible that under different conditions, such as in the behavioral discrimination task, small differences in context-specific coding parameters, including firing rates, could be amplified by contextual salience (such as footshock) and may be manifested in CA1. It remains to be seen whether the context specificity of CA3 coding will be transferred to CA1 under the conditions of behavioral discrimination.

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Materials and Methods
Figs. S1 to S6
Tables S1 to S3
References

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Identification of Active Edge Sites for Electrochemical H_2 Evolution from MoS_2 Nanocatalysts

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The identification of the active sites in heterogeneous catalysis requires a combination of surface sensitive methods and reactivity studies. We determined the active site for hydrogen evolution, a reaction catalyzed by precious metals, on nanoparticulate molybdenum disulfide (MoS_2) by atomically resolving the surface of this catalyst before measuring electrochemical activity in solution. By preparing MoS_2 nanoparticles of different sizes, we systematically varied the distribution of surface sites on MoS_2 nanoparticles on Au(111), which we quantified with scanning tunneling microscopy. Electrocatalytic activity measurements for hydrogen evolution correlate linearly with the number of edge sites on the MoS_2 catalyst.

Progress in the field of heterogeneous catalysis is often hampered by the difficulty of identifying the active site on a catalyst surface (*1*, *2*). In homogeneous catalysis, the active center is generally more clearly defined and quantified, with spectroscopic and mechanistic studies providing direct insight into reactive intermediates. Solid-state catalysts, however, commonly exhibit a variety of different surface sites that are difficult to identify and quantify; the scenario is further complicated when multiple sites work together in turning over a reaction. Identifying the most active site(s) is critical to designing and developing improved catalytic materials. Many useful in situ and ex situ experimental techniques, as well as computational methods, have been developed (*3*–*5*) to address this problem, but identifying the active site remains a challenging task.

In this study we used such methods to determine the active site of nanoparticulate MoS_2 for the hydrogen evolution reaction (HER), $2H^+ + 2e^- \rightarrow H_2$ (*6*, *7*), which is fundamentally important for a variety of electrochemical processes, fuel cells (as the reverse reaction), and solar H_2 production (water splitting), particularly where there is a need to replace precious metal catalysts such as Pt (*7*, *8*). In its bulk form, MoS_2 is a poor HER catalyst (*9*). Nanoparticulate MoS_2, however, is a more promising system; density functional theory (DFT) calculations indicate that the edges of MoS_2 nanoparticles are active for hydrogen evolution (*8*), but no previous experiments have shown this conclusively.

Nanoparticulate MoS_2 has been studied previously in an attempt to link activity to specific surface sites, in that MoS_2 is used industrially as a hydrodesulfurization (HDS) catalyst (*10*, *11*). Detailed insight has been gained from studies on simplified model systems in ultra-high vacuum (UHV) and by using computational methods (*12*–*15*), as well as from combining reactivity measurements and ex situ characterization of industrial catalyst samples (*10*, *11*, *16*). Structural studies on the MoS_2 catalyst have shown that it is composed almost entirely of flat polygons of S-Mo-S trilayers (*10*); depending on the synthesis conditions, these trilayers may stack in a graphite-like manner or remain as single trilayers. For single trilayers, two general kinds of surface sites exist—terrace sites, which are those on the basal plane, and edge sites, which lie at the edge of the nanoparticles. DFT studies suggest that the active site for HDS is on the edge of the MoS_2 nanoparticles. This result is supported by adsorption studies of thiophene using scanning tunneling microscopy (STM) (*17*). Despite numerous studies on this material, there is a call for studies that uniquely link the well-defined structures of the model system to catalytic activity under standard reaction conditions (*18*).

To provide an experimental elucidation of the active site for the HER, we prepared MoS_2 samples in UHV of deliberately chosen nanoparticulate morphologies such that the fractions of the terrace and edge sites were systematically varied, then characterized by STM. All of the MoS_2 samples in this study were synthesized on a clean Au(111) substrate by physical vapor deposition of Mo in a background of H_2S (*19*), followed by annealing, according to the approach in (*13*). Three samples were annealed at 400°C, two were annealed at 550°C, and a "blank" sample was synthesized without the deposition of Mo and annealed to 400°C. The Au(111) substrate serves to disperse the MoS_2 nanoparticles by its herringbone reconstruction and is not particularly active for the HER (*20*). To maintain discretely separated single trilayer particles, we purposely synthesized the samples with low area coverages of MoS_2, less than one-fourth ML (i.e., 0.25 $nm^2_{MoS2}/nm^2_{geometric}$).

Immediately after deposition, each sample was vacuum transferred to a second UHV chamber for STM imaging (Fig. 1). The crystallized, single-layered MoS_2 nanoparticles can be described as flat polygons with a conducting edge state, seen as bright lines along the particle perimeter. Comparison of representative images of samples annealed at 400°C (Fig. 1A) and 550°C (Fig. 1B) shows how particle size increased after sintering at the higher temperature. The particles annealed to 400°C are consistent with similarly prepared MoS_2 nanoparticles on Au(111) (*13*). Besenbacher *et al.* have shown that the dominant edge structure of MoS_2 nanoparticles is that of a sulfided Mo edge and that this edge is particularly favored by larger-sized particles (*12*, *18*). We also observe the predominance of the sulfided Mo-edge in our samples, regardless of annealing temperature. Thus, controlled sintering allows us to change the ratio of basal plane sites to edge sites without changing the nature of the edge. This sulfided (1 0 –1 0)

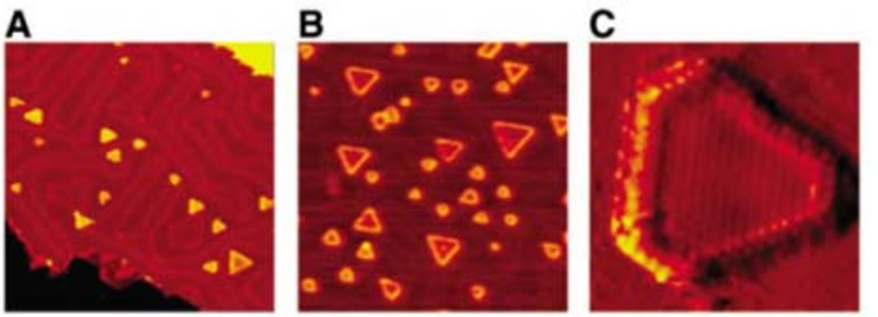

Fig. 1. A series of STM images of MoS_2 nanoparticles on Au(111). The particles exhibit the typical polygon morphology with conducting edge states and are dispersed on the Au surface irrespective of coverage and annealing temperature (400°C or 550°C). (**A**) Low coverage (0.06 $nm^2_{MoS2}/nm^2_{geom.}$), annealed to 400°C (470 Å by 470 Å, 1.2 nA, 4 mV). (**B**) High coverage (0.23 $nm^2_{MoS2}/nm^2_{geom.}$), annealed to 550°C (470 Å by 470 Å, 1.2 nA, 1.9 V). (**C**) Atomically resolved MoS_2 particle, from a sample annealed to 550°C, showing the predominance of the sulfided Mo-edge (*19*, *20*) (60 Å by 60 Å, 1.0 nA, 300 mV).

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Mo-edge is the same structure predicted by DFT calculations to be the active site for H_2 evolution (*8*).

After imaging, we transfer the samples from UHV into an electrochemical cell to measure HER activity (*21*). Polarization curves ($i - E$) within a cathodic potential window, and corresponding Tafel plots (log $i - E$), are shown in Fig. 2. Current densities are normalized to the geometric area of the exposed face of all samples.

The most inherent measure of activity for the HER is the exchange current density, i_0 (*6*, *7*, *22*, *23*), which is determined by fitting $i - E$ data to the Tafel equation (*6*), yielding Tafel slopes of 55 to 60 mV/decade and exchange current densities in the range of 1.3×10^{-7} to 3.1×10^{-7} A/cm$^2_{geometric}$ for all MoS_2 samples (table S1). In Fig. 3, we plot the exchange current density for each sample versus two sample parameters, the MoS_2 area coverage (Fig. 3A), and the MoS_2 edge state length (Fig. 3B). The data points fall on a straight line only when plotted versus edge length. Although the points show some scatter around this trend, they are described by a best-fit linear relation with a slope of 1.67×10^{-20} A/nm$_{MoS2\text{-}edge}$.

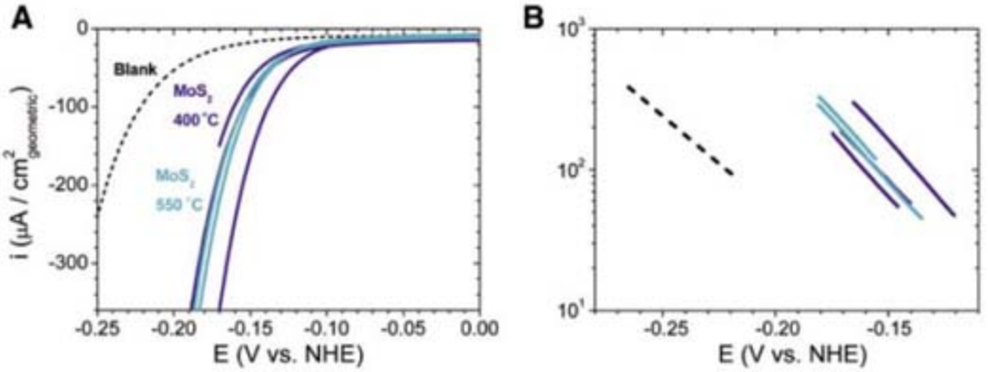

Fig. 2. Polarization curves and Tafel plots in a cathodic potential window for the five different MoS_2 samples as well as a blank sample. Samples annealed to 400°C are dark blue, samples annealed to 550°C light blue. (**A**) Polarization curve showing H_2 evolution on all samples. (**B**) Tafel plot (log current versus potential). All of the MoS_2 samples have Tafel slopes of 55 to 60 mV per decade irrespective of annealing temperature and coverage. Sweep rate: 5 mV/s.

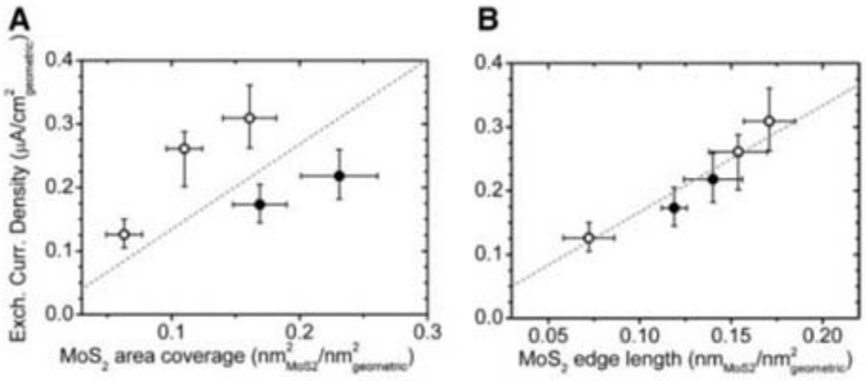

Fig. 3. Exchange current density versus (**A**) MoS_2 area coverage and (**B**) MoS_2 edge length. In both figures, open circles are samples annealed to 400°C, filled circles are samples annealed to 550°C. The exchange current density does not correlate with the area coverage of MoS_2, whereas it shows a linear dependence on the MoS_2 edge length. Exchange current densities are extracted from the Tafel plot in Fig. 2. The edge length was measured on all imaged particles and normalized by the imaged area.

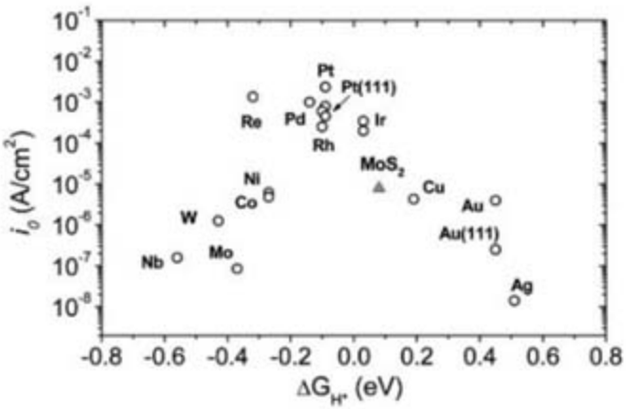

Fig. 4. Volcano plot of the exchange current density as a function of the DFT-calculated Gibbs free energy of adsorbed atomic hydrogen for nanoparticulate MoS_2 and the pure metals (*23*). As seen, MoS_2 follows the same trend as the pure metals. The MoS_2 exchange current density is normalized to the atomic site density of Pt for comparison. Samples are polycrystalline unless otherwise noted.

Because the rate of reaction is directly proportional to the number of edge sites for all samples, regardless of particle size, we conclude that the edge site is indeed the active site (*24*). Bearing this in mind, we note in Fig. 3A that the exchange current densities of the samples sintered at 550°C are significantly lower than those prepared at 400°C, per MoS_2 coverage, exactly as one would expect considering that the sintered samples have less edge length per area of MoS_2.

We also compared nanoscale MoS_2 to other materials that catalyze the HER on a per active site basis (*1*). For this direct site-to-site comparison, we used the 1.5×10^{15} sites/cm^2 for the Pt(111) face as the basis for comparison as Pt is the archetypical HER catalyst (*25*). An exchange current density of 4.5×10^{-4} A/cm^2 for this face (*26*) yields a turnover frequency (TOF) of 0.9 s^{-1} (table S2). In general, TOFs of transition metals range over 10 orders of magnitude (Hg, for instance, has a TOF as low as ~10^{-9} s^{-1}) (*22*). Given the slope in Fig. 3B, we have calculated the TOF of the MoS_2 edge to be 0.02 s^{-1}, indeed in the high range of TOFs for metals.

For further insight into the catalytic nature of the MoS_2 edge, we have added our data for nanoparticulate MoS_2 to a recent version of the volcano-type relations observed for HER catalysts (Fig. 4), in this case for the Gibbs free energy for atomic hydrogen adsorption (ΔG_H) (*22*, *23*). These volcano relations ultimately reflect the Sabatier principle, which accounts for optimal surfaces as ones that exhibit moderate binding energies of reaction intermediates, hydrogen adsorption in the case of the HER. In Fig. 4, the exchange current density is shown as a function of the DFT-calculated free energy of adsorption of hydrogen, which was recently determined to be +0.08 eV for the MoS_2 edge (*8*). To add MoS_2 to this figure, we converted the TOF of nanoparticulate MoS_2 to its exchange current density per 1.5×10^{15} sites/cm^2, which yields 7.9×10^{-6} A/cm^2 (table S2). This value surpasses those of the common metals and lies just below those of the precious Pt-group metals. When plotting this experimentally determined activity of the edge site versus its DFT-calculated ΔG_H (*8*), we see that it follows the volcano trend (*23*). This agreement validates the predictive capability of this DFT model as well as its applicability beyond metal catalysts.

After identifying the active site and comparing it with typical metal catalysts, we may

consider how to improve its activity. The DFT-calculated ΔG_H of the MoS_2 edge site is slightly positive at +0.08 eV, with calculations suggesting an H coverage of only one-quarter on the edge under operating conditions (*8*). Thus, only 1 in 4 edge atoms evolves molecular H_2 at a given time, unlike Pt(111) which operates at a H-coverage of ~1 ML (*7*, *26*, *27*). If all MoS_2 edge sites could be made to adsorb H, activity could be increased by a factor of 4. This might be accomplished by appropriately tuning the electronic structure of the edge to increase the bond strength of the adsorbed H (*23*). Such a modification could simultaneously improve the inherent turnover of each edge site, further improving the overall activity of the material toward that of Pt-group metals.

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Materials and Methods
SOM Text
Fig. S1
Tables S1 and S2
References

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Understanding Reactivity at Very Low Temperatures: The Reactions of Oxygen Atoms with Alkenes

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A remarkable number of reactions between neutral free radicals and neutral molecules have been shown to remain rapid down to temperatures as low as 20 kelvin. The rate coefficients generally increase as the temperature is lowered. We examined the reasons for this temperature dependence through a combined experimental and theoretical study of the reactions of $O(^3P)$ atoms with a range of alkenes. The factors that control the rate coefficients were shown to be rather subtle, but excellent agreement was obtained between the experimental results and microcanonical transition state theory calculations based on ab initio representations of the potential energy surfaces describing the interaction between the reactants.

Application of the CRESU technique (*1*) has shown that a surprising number of bimolecular reactions between neutral gas-phase species are rapid at very low temperatures. To date, rate coefficients for some 45 neutral-neutral reactions have been measured (*2*, *3*), in some cases at temperatures as low as 13 K (*4*). All have rate coefficients at 298 K [k(298 K)] that are equal to or exceed ~10^{-11} cm^3 $molecule^{-1}$ s^{-1}. Moreover, the general trend with temperature is for the rate coefficients to increase as the temperature is lowered.

These observations have led to a reevaluation of the chemistry that occurs in the cold cores (10 to 20 K) of dense, dark interstellar clouds (ISCs), where the majority of interstellar molecules have been identified. Although sequences of ion-molecule reactions initiated by the cosmic ray–induced ionization of H_2 clearly play a central role in this chemistry, neutral-neutral reactions are now expected to be more important than previously thought (*5*). Unfortunately, the kinetic database required for detailed astrochemical modeling (*6*) is still far from complete. There are many reactions that may occur in ISCs, such as those between pairs of unstable species, for which low-temperature kinetic data neither exist nor are likely to be obtained in the foreseeable future. Theoretical or semi-empirical methods of estimating these rate coefficients are therefore desirable.

Several complementary theoretical treatments (*7*–*9*) have been advanced to explain the observed negative temperature dependences of rate coefficients for radical-radical reactions, principally on the basis of the notion of adiabatic capture of the reactants via long-range attractive forces. Although they differ in their details, these treatments all predict large rate coefficients at very low temperatures for radical-radical reactions, where there is generally no barrier on the minimum energy reaction path. In the case of radical-molecule reactions, a key issue is whether a potential energy barrier exists along the minimum energy path from reactants to products: either a real barrier (i.e., a maximum above the energy of the separated reactants) or a "submerged" barrier corresponding to a maximum along the minimum energy path between the shallow minimum associated with a prereaction complex and the products (see below). Further theoretical work, particularly by Georgievskii and Klippenstein (*10*, *11*), has shown that a submerged barrier can serve as a second inner transition state (or bottleneck), because the internal states at this smaller interreactant separation are more widely spaced than at the outer, capture transition state. In these circumstances, the rate of reaction falls below that predicted by capture theories, and a version of microcanonical transi-

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tion state theory must be applied to make accurate predictions of the rate coefficients and their variation with temperature.

From the standpoint of modeling ISC chemistry, it would be useful to have semi-empirical methods to predict which reactions might remain fast at very low temperatures. In this spirit, Smith *et al.* (*3*) examined the data available for reactions between atomic and molecular free radicals and unsaturated hydrocarbons, seeking a framework to predict whether particular radical-molecule reactions would be rapid enough ($k > 10^{-11}$ cm^3 molecule^{-1} s^{-1}) to influence the abundances of chemical species in ISCs at temperatures ≤ 20 K. Their first method, based purely on an examination of the experimental data amassed to date, led them to propose that a radical-molecule reaction is likely to be rapid at 10 to 20 K if its room-temperature rate coefficient, k(298 K), is greater than ~5×10^{-12} cm^3 molecule^{-1} s^{-1} and its activation energy, E_{act}, around 298 K is zero or negative. Conversely, reactions with k(298 K) $< 5 \times 10^{-12}$ cm^3 molecule^{-1} s^{-1} and $E_{act} > 0$ are likely to be too slow at 10 to 20 K to be of importance in ISC chemistry.

Their second approach to the problem of predicting the presence of a real potential barrier to reaction rests on the notion that such barriers are often the product of an avoided curve crossing between two states: the reactant ground state, which correlates with a product ionic state, and an excited reactant ionic state, which correlates with the product ground state (*12*). The barrier that arises depends especially on the energy of the reactant ionic state, which depends in turn on the difference between the ionization energy (I.E.) of the molecule and the electron affinity (E.A.) of the radical, corresponding to virtual electron transfer between the molecule (the donor) and the radical (the acceptor). If (I.E. – E.A.) is small, in the presence of long-range attraction this barrier can be submerged below the reactant energy.

After analyzing the rate coefficients and their temperature dependences for a large number of such reactions, most of which had been studied at very low temperatures, Smith *et al.* (*3*) proposed that, when (I.E. – E.A.) is greater than 8.75 eV, the reaction is likely to possess a true barrier and will therefore become negligibly slow at 20 K. On the other hand, reactions with (I.E. – E.A.) <8.75 eV are likely to be characterized by, at most, inner barriers that are submerged below the asymptotic reactant energy. These latter reactions will be rapid at 20 K and of potential importance in ISC chemistry. Smith *et al.* further noted that the reactions between oxygen atoms in their ^{3}P electronic ground state and simple unsaturated hydrocarbons have values of (I.E. – E.A.) that bridge the critical value of 8.75 eV (see Table 1) and would therefore provide an important test of their hypothesis.

To test these ideas, we measured rate coefficients for the reactions between O(^{3}P) atoms and the alkenes listed in Table 1 in a continuous-flow CRESU apparatus (*4*). Experiments were performed at temperatures between 23 and 298 K with the use of different convergent-divergent Laval nozzles and carrier gases. Details of the experimental conditions are given in table S1.

For each kinetic experiment, in addition to the carrier gas (which was present in large excess), three gases were generally included in the supersonic flow: the alkene whose reaction was being studied; NO_2, which was photolyzed at 355 nm by pulses from a frequency-tripled Nd:YAG laser to produce O(^{3}P) atoms; and NO. Chemiluminescence from excited NO_2, formed in the association of O(^{3}P) with NO, served as a marker for the oxygen atom concentrations and decayed as the atoms were consumed in reaction with the alkene. Figure 1 shows two such decay traces, one recorded at 298 K and one at 39 K, both with a small concentration of *iso*-butene present in the gas mixture. Each trace was fitted to a single exponential curve to yield a pseudo–first-order rate coefficient (k_{1st}) for removal of O(^{3}P) atoms under the conditions of that particular experiment.

In each series of experiments, values of k_{1st} were determined for several different concentrations of alkene and the second-order rate coefficient (k_{2nd}) for the reaction of that alkene with O(^{3}P) atoms at that particular temperature was derived, as shown in Fig. 1, from the gradient of a plot of k_{1st} versus the concentration of the alkene. The results of these experiments are summarized in Fig. 2. Full quantitative details are given in table S1, and the errors in the experimental data are shown in fig. S1.

Figure 2 shows the existence of three distinct kinds of kinetic behavior for O(^{3}P) + alkene reactions. *Iso*-butene, *cis*-butene, and *trans*-butene have the lowest I.E.s of the alkenes used in our experiments, and (I.E. – E.A.) is less than 8 eV for the reactions of O(^{3}P) with these alkenes.

Table 1. In the first three lines, rate coefficients, k(298 K), and activation energies, E_{act}, from Cvetanovic (*13*) are compared with (I.E. – E.A.) for the reactions of O(^{3}P) atoms with alkenes; the electron affinity of the O(^{3}P) atoms is 1.46 eV. The last two lines give CASPT2 energies on the minimum-energy path (all relative to the energy of the separated reactants in their ground state): E_{min}, electronic + zero-point energy at the minimum of the "prereaction complex"; E_{max}, electronic + zero-point energy at the inner transition state. Bracketed entries are for the excited-state potential; where there are upper and lower entries, the upper entries are for addition at a terminal site, lower entries for addition at a central site.

	Ethene	Propene	1-Butene	*cis*-Butene	*iso*-Butene	*trans*-Butene
k(298) (10^{-11} cm^3 molecule^{-1} s^{-1})	0.073	0.40	0.42	1.8	1.7	2.2
E_{act} (kJ mol^{-1})	6.65	2.3	2.9	−1.2	−0.1	−0.1
(I.E. – E.A.) (eV)	9.05	8.27	8.09	7.65	7.76	7.64
E_{min} (kJ mol^{-1})	−1.3 (−0.9)	−4.7	−5.2	−5.7	−5.9	−6.3
E_{max} (kJ mol^{-1})	5.4 (11.8)	0.9 (5.9) 1.5 (8.0)	−0.5 (5.0) 0.1 (5.9)	−2.2 (3.3)	−2.5 (2.6) −1.3 (6.4)	−3.1 (2.3)

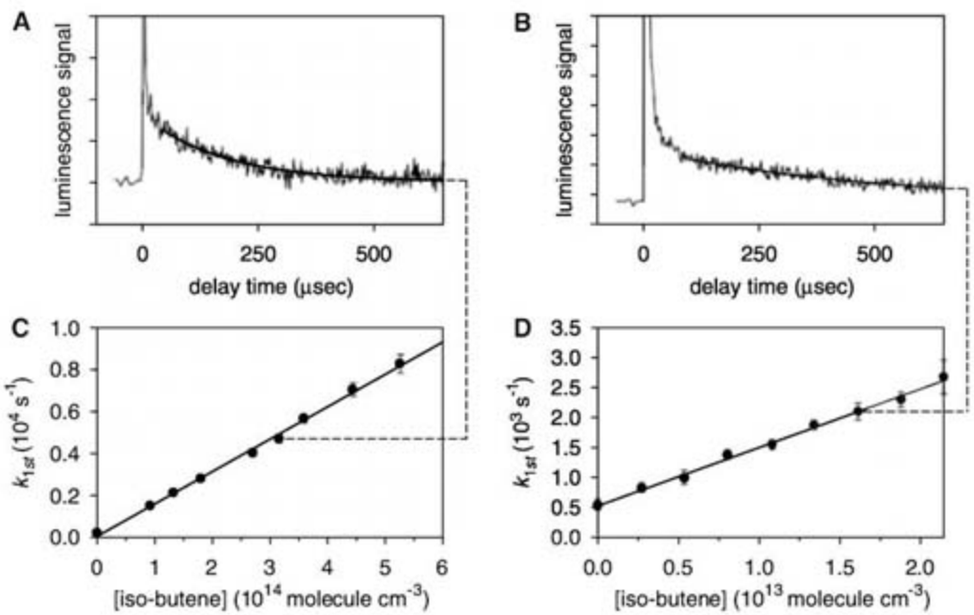

Fig. 1. (**A** and **B**) Fitted decays of the chemiluminescence from NO_2 observed in mixtures containing (A) 3.20×10^{14} molecules cm^{-3} *iso*-butene at 298 K and (B) 1.62×10^{13} molecules cm^{-3} *iso*-butene at 39 K. (**C** and **D**) Plots of the pseudo–first-order rate coefficients obtained from such experiments plotted against the concentration of *iso*-butene at (C) 298 K and (D) 39 K.

Moreover, the values of k(298 K) exceed 10^{-11} cm^3 molecule^{-1} s^{-1}, with the rate coefficients showing a zero or slightly negative dependence on temperature between 300 and 500 K. Below 298 K, as expected, the rate coefficients for these reactions show a monotonic increase as the temperature is lowered, reaching values of ~10^{-10} cm^3 molecule^{-1} s^{-1} at 23 K, the lowest temperature studied here.

The other limit of behavior is exhibited in the O(^{3}P) + C_2H_4 reaction. Our result of k(298 K) = 7.4×10^{-13} cm^3 molecule^{-1} s^{-1} agrees very well with literature values (*13*), but only upper limits could be obtained at lower temperatures, k(39 K) < 1.6×10^{-13} cm^3 molecule^{-1} s^{-1}. The appreciable activation energy for this reaction (*13*, *14*) and its relatively large value of (I.E. – E.A.) both suggest that this reaction should be very slow at the temperatures accessible in a CRESU apparatus (and present in dense ISCs). The existence of an appreciable real barrier on the potential for this reaction, as shown in Fig. 3, is also supported by previous ab initio calculations (*15*).

The reactions of O(^{3}P) atoms with 1-butene and propene provide the most demanding test of the hypotheses made by Smith *et al.* (*3*). The rate coefficients for both reactions at 298 K are close to the value of k(298 K) = 5×10^{-12} cm^3 molecule^{-1} s^{-1}, which Smith *et al.* proposed might separate rapid from slow low-temperature reactions. Moreover, the rate coefficients exhibit small positive dependences on temperature, represented by the activation energies given in Table 1 and the curves in Fig. 2. On the other hand, their values of (I.E. – E.A.) are less than, although not much less than, 8.75 eV. The rate coefficients below 298 K measured in the present work increase as the temperature is lowered. The variation of the rate coefficients with temperature for both these reactions is similar to that found for the reaction of CN with C_2H_6 (*16*), which has recently been reproduced in microcanonical transition state theory calculations (*11*) that allow for the presence of both an outer and inner transition state.

To understand more deeply both the observed kinetic behavior of the reactions of O(^{3}P) atoms with alkenes and the origins of the partly successful correlations of the rate coefficients and the temperature dependence with (I.E. – E.A.), we carried out calculations similar to those on CN with C_2H_6 (*11*) and on OH with C_2H_4 (*10*). These calculations consider only the rate of formation of the strongly bound triplet addition complex between O(^{3}P) and an alkene. In general, this complex is either stabilized by collision with a third body or dissociates to exothermic reaction products (*13*), such as CH_2CHO + H or CH_3 + HCO when O(^{3}P) atoms react with C_2H_4. The focus of the present calculations is the accurate treatment of both the inner and outer transition state regions on the basis of high-level ab initio studies of the potential energy surface. These properties were determined with second-order multireference perturbation theory (CASPT2) using the MOLPRO quantum chemistry package (*17*).

For the O(^{3}P) + alkene reactions, there are multiple inner transition states correlating with different addition sites and with different electronic states. In particular, the O(^{3}P) atom can add to either carbon atom of the π bond, with the terminal addition (i.e., to the C atom of the $=CH_2$ group) generally being preferred. Also, two of the three orientations for the lone pair orbital on the oxygen atom correlate with strong bonding in the complex. However, the barrier for the first excited state is always above the energy of the reactants and thus has little effect at low temperatures.

The electronic dynamics are assumed to be adiabatic, and the ground and excited electronic states at the inner transition state are correlated with the long-range spin-orbit states (fig. S2). The inner, $N^{\dagger}_{\text{inner},i}$, and outer, $N^{\dagger}_{\text{outer},i}$, transition state number of states for a given electronic state i are related to an effective number of states, $N^{\dagger}_{\text{eff},i}$, in Eq. 1 (*10*, *11*, *18*)

$$\frac{1}{N^{\dagger}_{\text{eff},i}} = \frac{1}{N^{\dagger}_{\text{inner},i}} + \frac{1}{N^{\dagger}_{\text{outer},i}} \qquad (1)$$

For each of the reactions studied here, a rate coefficient for long-range capture of ~4 to 5×10^{-10} cm^3 molecule^{-1} s^{-1} is predicted at 20 K, with a modest (~$T^{1/6}$) increase with temperature. The adiabatic electronic assumptions reduce the low-temperature rate coefficients by a factor of

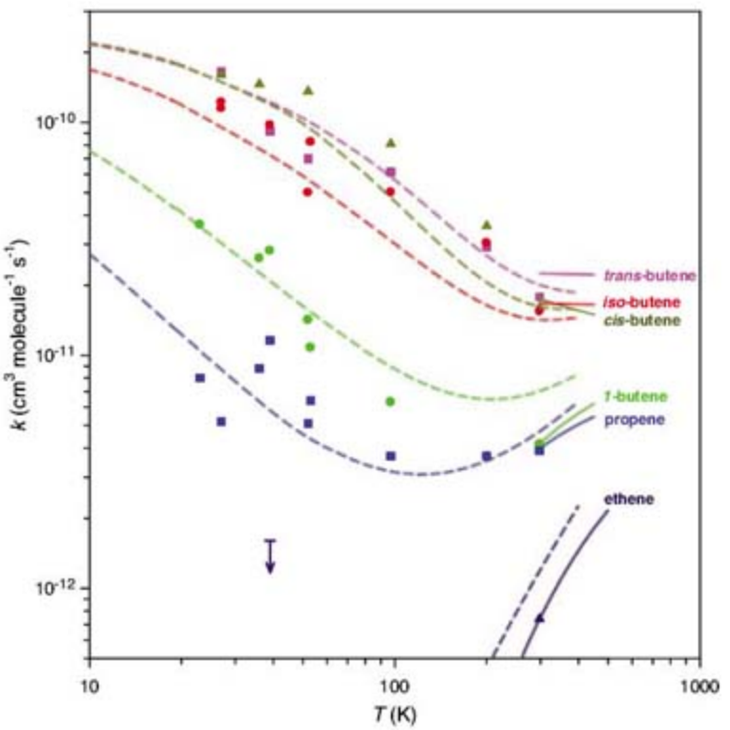

Fig. 2. The points show the experimentally determined values of the rate coefficients for the reactions of O(^{3}P) atoms with alkenes at different temperatures, and the dashed lines show the results of the theoretical calculations. The solid lines to the right of the diagram represent the Arrhenius expressions recommended by Cvetanovic (*13*) to fit kinetic data between 300 and 500 K.

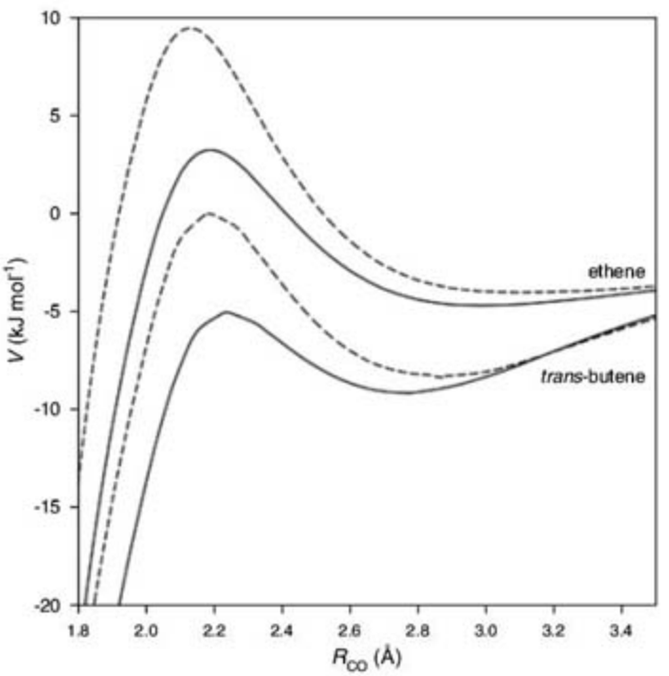

Fig. 3. Curves showing the variation of potential energy along the minimum energy paths for O(^{3}P) + ethene (upper pair of curves) and O(^{3}P) + *trans*-butene (lower pair of curves), calculated using the CASPT2 method. The ground and first excited electronic states are shown by the solid and dashed lines, respectively. All curves show minima associated with the prereaction complex and maxima associated with real or submerged barriers. The zero of energy is set at the energy of the separated reactants.

3/5, to 2.5 to 3 × 10^{-10} cm^3 $molecule^{-1}$ s^{-1} at 20 K, which is within a factor of 2 of the measured rate coefficients for *cis*- and *trans*-butene.

The CASPT2 predictions for the saddle point energies, given in Table 1, correlate with the expectations, discussed earlier, based on the different values of (I.E. – E.A.). For ethene, the positive barrier leads to a low reactivity at low temperature. Propene and 1-butene are near ideal intermediate cases, with barriers within 1 kJ mol^{-1} of the reactants. For propene, the inner transition state strongly affects the rate coefficient even at 20 K, but the barrier is just low enough that, with tunneling, the rate coefficient does not decay to small values at low temperatures. For 1-butene, the rate coefficient rises with decreasing temperature but is still an order of magnitude below the long-range capture value at 20 K. For the other butenes, the ground-state barriers are strongly submerged (i.e., by 2 to 3 kJ mol^{-1}) and the addition rates increase rapidly with decreasing temperature, exceeding 10^{-10} cm^3 $molecule^{-1}$ s^{-1} by 20 K. The changing nature of the reaction with changing (I.E. – E.A.) is illustrated by the plots in Fig. 3 of the minimum energy path potentials as a function of the CO separation for the ground and first excited states in the $O(^3P)$ + ethene and $O(^3P)$ + *trans*-butene reactions.

The calculated rate coefficients for addition arising from the present two–transition state model are illustrated as dashed lines in Fig. 2. The calculations have been extended down to 1 K, and the results for the range from 10 to 1 K are given in fig. S1. For $O(^3P)$ + propene, the predicted rate coefficients are highly sensitive to the energy of the inner saddle point with a factor of 2 variation at 100 K for a 0.7 kJ mol^{-1} change. The CASPT2 predicted barrier was adjusted downward by 0.6 kJ mol^{-1} to obtain optimum agreement with experiment. For consistency, this adjustment was applied to the other reactions as well. For the butene cases, this adjustment has an insignificant effect on the predictions.

The remarkably good agreement between the theoretical predictions and experimental observations strongly validates the present two–transition state model. The calculations show that both the inner and outer transition states have an effect on the reaction kinetics throughout the 20 to 400 K range. At low temperatures the outer transition state dominates, whereas at high temperatures the inner transition state dominates. The increasing importance of the inner transition state with increasing temperature causes the negative temperature dependence between 200 and 20 K. The $O(^3P)$ + C_2H_4 reaction, where the positive barrier for the inner transition state dominates the kinetics at all temperatures, provides the only exception. The accurate treatment of both transition-state regions is a key prerequisite to understanding and predicting the kinetics of these reactions at low temperatures.

The agreement between experiment and theory found for the $O(^3P)$ + alkene reactions suggests two ways in which CASPT2 calculations of barrier heights might be used to estimate the possible importance in ISCs of other reactions between radicals and unsaturated molecules, depending on whether experimental information is available for the kinetics of the reaction in question—for example, a measured value of the rate coefficient at room temperature. If so, uncertainty in the ab initio calculations of the inner barrier height, which experience suggests might amount to a few kJ mol^{-1}, could be reduced by comparing theoretical and experimental values of the room-temperature rate coefficient so as to tune the potential energy at the inner transition state, and thereby improve the theoretical estimate of the low-temperature rate coefficients. As described above, we adopted this method with the use of the data for $O(^3P)$ + propene, although we found that the adjustment needed was quite small. In cases where no kinetic data exist, such as for the reactions of radicals with carbon chains (*19*), more reliance will have to be placed on the theory, guided by the success of the present calculations, or on the semi-empirical arguments concerning the value of (I.E. – E.A.). At the very least, these methods should provide guidance at the order-of-magnitude level, which is itself valuable at the present level of astrochemical modeling.

References and Notes

1. The acronym CRESU stands for Cinétique de Réaction en Ecoulement Supersonique Uniforme, or Reaction Kinetics in Uniform Supersonic Flow. The technique was originally developed by Rowe and his co-workers for the study of ion-molecule reactions (*20*).
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Supporting Online Material

www.sciencemag.org/cgi/content/full/317/5834/102/DC1
Materials and Methods
SOM Text
Figs. S1 and S2
Tables S1 and S2
References

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Long-Lived Giant Number Fluctuations in a Swarming Granular Nematic

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Coherently moving flocks of birds, beasts, or bacteria are examples of living matter with spontaneous orientational order. How do these systems differ from thermal equilibrium systems with such liquid crystalline order? Working with a fluidized monolayer of macroscopic rods in the nematic liquid crystalline phase, we find giant number fluctuations consistent with a standard deviation growing linearly with the mean, in contrast to any situation where the central limit theorem applies. These fluctuations are long-lived, decaying only as a logarithmic function of time. This shows that flocking, coherent motion, and large-scale inhomogeneity can appear in a system in which particles do not communicate except by contact.

Density is a property that one can measure with arbitrary accuracy for materials at thermal equilibrium simply by increasing the size of the volume observed. This is because a region of volume V, with N particles on average, ordinarily shows fluctuations with standard deviation ΔN proportional to $\sqrt{N}$, so that fluctuations in the number density go down as $1/\sqrt{V}$. Liquid crystalline phases of active or self-propelled particles (*1*–*4*) are different, with ΔN predicted (*2*–*5*) to grow faster than $\sqrt{N}$ and as fast as N in some cases (*5*), making density an ill-defined quantity even in the limit of a large system. These predictions show that flocking,

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coherent motion, and giant density fluctuations are intimately related consequences of the orientational order that develops in a sufficiently dense grouping of self-driven objects with anisotropic body shape. This has substantial implications for biological pattern formation and movement ecology (*6*): The coupling of density fluctuations to alignment of individuals will affect populations as diverse as herds of cattle, swarms of locusts (*7*), schools of fish (*8*, *9*), motile cells (*10*), and filaments driven by motor proteins (*11*–*13*).

We report here that persistent giant number fluctuations and the coupling of particle currents to particle orientation arise in a far simpler driven system, namely, an agitated monolayer of rodlike particles shown in (*14*) to exhibit liquid crystalline order. These fluctuations have also been observed in computer simulations of a simple model of the flocking of apolar particles by Chaté *et al.* (*15*). The rods we used were cut to a length $l = 4.6 \pm 0.16$ (SEM) mm from copper wire of diameter $d = 0.8$ mm. The ends of the rods were etched to give them the shape of a rolling pin. The rods were confined in a quasi-two-dimensional cell 1 mm tall and with a circular cross-section 13 cm in diameter. The cell was mounted in the horizontal plane on a permanent magnet shaker and vibrated vertically at a frequency $f = 200$ Hz, with an amplitude, A, between 0.025 and 0.043 mm. The resultant dimensionless acceleration $\Gamma = (4\pi^2 f^2 A)/g$, where g is the acceleration due to gravity, varies between $\Gamma = 4$ and $\Gamma = 7$. We varied the total number of particles in the cell, N_{total}, between 1500 and 2820. N_{total} in each instance was counted by hand. The area fraction, ϕ, occupied by the particles is the total projected area of all the rods divided by the surface area of the cell. ϕ varies from 35% to 66%. Our experimental system is similar to those used to study the phase behavior of inelastic spheres (*16*, *17*). Galanis *et al.* (*18*) shook rods in a similar setup, albeit with much less confinement in the vertical direction. The particles were imaged with a digital camera (*19*).

The rods gain kinetic energy through frequent collisions with the floor and the ceiling of the cell. Because the axes of the particles are almost always inclined to the horizontal, these collisions impart or absorb momentum in the horizontal plane. Collisions between particles conserve momentum but also drive horizontal motion by converting vertical motion into motion in the plane. Interparticle collisions as well as particle-wall collisions are inelastic, and all particle motion would cease within a few collision times if the vibrations were switched off. The momentum of the system of rods is not conserved either, because the walls of the cell can absorb or impart momentum. The rods are apolar; that is, individual particles do not have a distinct head and tail that determine fore-aft orientation or direction of motion and can form a true nematic phase. The experimental system thus has all the physical ingredients of an active nematic (*1*–*4*).

The system is in a very dynamic steady state, with particle motion (movie S1) organized in macroscopic swirls. Swirling motions do not necessarily imply the existence of giant number fluctuations (*20*, *21*); however, particle motions in our system generate anomalously large fluctuations in density. Figure 1A shows a typical instantaneous configuration, and the Fig. 1B inset shows the orientational correlation function $G_2(r) = \langle \cos 2(\theta_i - \theta_j) \rangle$, where i,j run over pairs of particles separated by a distance r and oriented at angles θ_i and θ_j with respect to a reference axis. The angle brackets denote an average over all such pairs and about 150 images spaced 15 s apart in time. The data in the inset show that the systems with $N_{total} = 2500$ and $N_{total} = 2820$ display quasi–long-ranged nematic order, where $G_2(r)$ decays as a power of the separation, r. On the other hand, the system with $N_{total} = 1500$ shows only short-ranged nematic order, with $G_2(r)$ decaying exponentially with r. Details of the crossover between these two behaviors can be found in [Supporting Online Material (SOM) text]. Autocorrelations of the density field as well as of the orientation of a tagged particle decay to zero on much shorter time scales (SOM text), so we expect these images to be statistically independent. To quantify the number fluctuations, we extracted from each image the number of particles in subsystems of

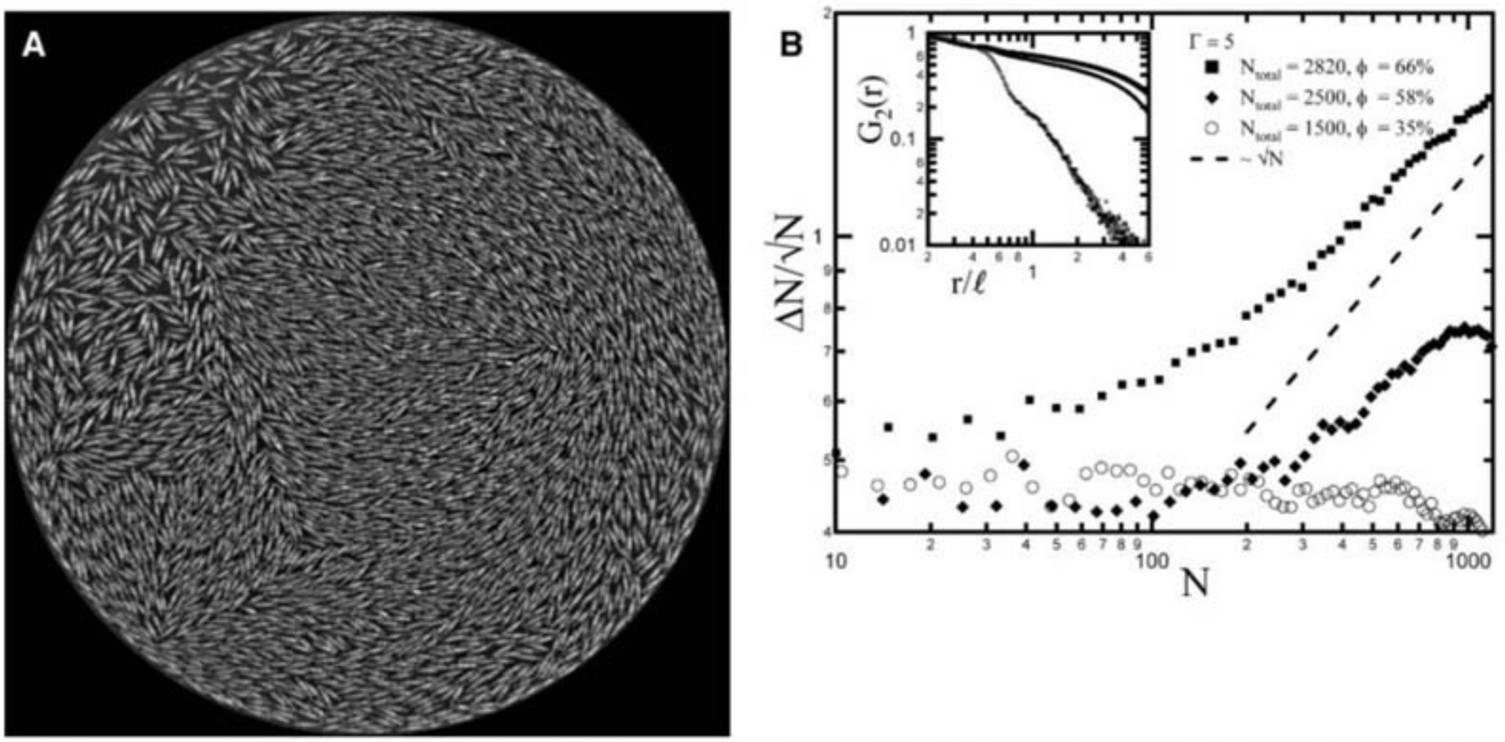

Fig. 1. Giant number fluctuations in active granular rods. (**A**) A snapshot of the nematic order assumed by the rods. There are 2820 particles (counted by hand) in the cell (area fraction is 66%) being sinusoidally vibrated perpendicular to the plane of the image, at a peak acceleration of $\Gamma = 5$. The sparse region at the top between 10 and 11 o'clock is an instance of a large density fluctuation. These take several minutes to relax and form elsewhere. (**B**) The magnitude of the number fluctuations (quantified by ΔN and normalized by $\sqrt{N}$) against the mean number of particles, for subsystems of various sizes. The number fluctuations in each subsystem are determined from images taken every 15 s over a period of 40 min (*19*). The squares represent the system shown in (A). It is a dense system where the nematic order is well developed. The magnitude of the scaled number fluctuations decreases in more dilute systems, where the nematic order is weaker (SOM text). Deviations from the central limit theorem result are still visible at an area fraction ≅ 58% (diamonds) but not at an area fraction ≅ 35% (circles). (Inset) The nematic-order correlation function as a function of spatial separation.

different size, defined by windows ranging in size from $0.1l$ by $0.1l$ to $12l$ by $12l$. From a series of images we determined, for each subsystem size, the average N and the standard deviation, ΔN, of the number of particles in the window. For any system in which the number fluctuations obey the conditions of the central limit theorem (*22*), $\Delta N/\sqrt{N}$ should be a constant, independent of N. Figure 1B shows that, when the area fraction ϕ is large, $\Delta N/\sqrt{N}$ is not a constant. Indeed, for big enough subsystems, the data show giant fluctuations, ΔN, in the number of particles, growing far more rapidly than $\sqrt{N}$ and consistent with a proportionality to N. For smaller average number density, where nematic order is poorly developed, this effect disappears, and $\Delta N/\sqrt{N}$ is independent of N, as in thermal equilibrium systems. The roll-off in $\Delta N/\sqrt{N}$ at the highest values of N is a finite-size effect: For subsystems that approach the size of the entire system, large number fluctuations are no longer possible because the total number of particles in the cell is held fixed.

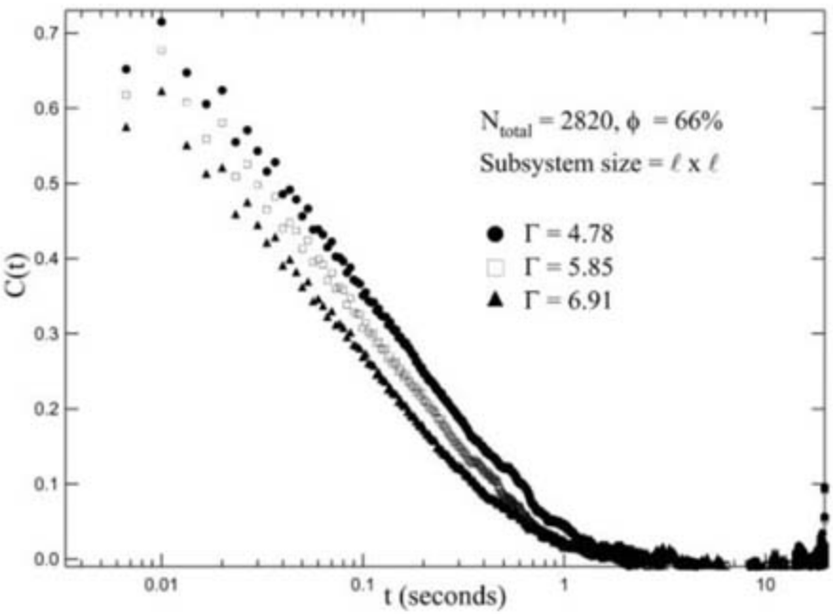

Fig. 2. The logarithmic dependence of the local density autocorrelation, $C(t) = \langle\varphi(0)\varphi(t)\rangle$ [$\varphi(t)$ is the deviation from the mean of the instantaneous number density of particles], is a direct consequence (SOM text), and hence a clear signature, of the large density fluctuations in the system. It is remarkable that such a local property reflects the dynamics of the entire system so strongly. It is seen that increasing Γ shortens the decay time. This is consistent with the fact that the magnitude of the giant number fluctuations grows with the nematic order (SOM text).

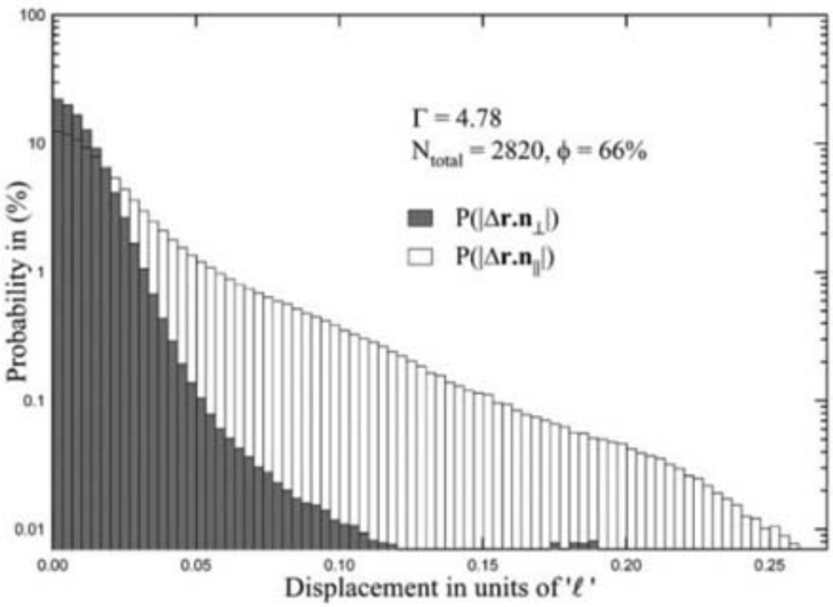

Fig. 3. The microscopic origin of the macroscopic density fluctuations. The probability distribution of the magnitude of the displacement along and transverse to the particle's long axis over an interval of 1/300 of a second shows that short time motion of the rods is anisotropic even at the time scale of the collision time. This anisotropy is explicitly forbidden in equilibrium systems by the equipartition theorem.

We examined a subsystem of size l by l (i.e., one rod length on a side) and obtained a time series of particle number, $N(t)$, by taking images at a frame rate of 300 frames per s. From this we determined the temporal autocorrelation, $C(t)$, of the density fluctuations. $C(t)$ decays logarithmically in time (Fig. 2), unlike the much more rapid t^{-1} decay of random, diffusively relaxing density fluctuations in two dimensions. Thus, the density fluctuations are not only anomalously large in magnitude but also extremely long-lived. Indeed, these two effects are intimately related: An intermediate step in the theoretical argument (*5*) that predicts giant number fluctuations shows that density fluctuations at a wave number q have a variance proportional to q^{-2} and decay diffusively. This leads to the conclusion (SOM text) that in the time regime intermediate between the times taken for a density mode to diffuse a particle length and the size of the system, the autocorrelation function of the local density decays only logarithmically in time. Although the observations agree with the predicted logarithmic decay, we cannot as yet make quantitative statements about the coefficient of the logarithm. We note that the size of subsystem is below the scale of subsystem size at which the standard deviation has become proportional to the mean. In flocks and herds as well, measuring the dynamics of local density fluctuations will yield crucial information regarding the entire system's dynamics and can be used to test the predictions of Toner and Tu (*2*, *3*).

What are the microscopic origins of the giant density fluctuations? Both in active and in equilibrium systems, particle motions lead to spatial variations in the nematic ordering direction. However, in active systems alone, such bend and splay of the orientation are predicted (*5*) to select a direction for coherent particle currents. These curvature-driven currents in turn engender giant number fluctuations. We find qualitative evidence for curvature-induced currents in the flow of particles near topological defects (SOM text). In the apolar flocking model of (*15*), particles move by hopping along their axes and then reorienting, with a preference to align parallel to the average orientation of particles in their neighborhood. Requiring that the hop be along the particle axis was sufficient to produce giant number fluctuations in the nematic phase of the system. It was further suggested in (*15*) that the curvature-induced currents of (*5*), although not explicitly put into their simulation, must emerge as a macroscopic consequence of the rules imposed on microscopic motion. This suggestion is substantiated by the work of Ahmadi *et al.* (*23*), who started from a microscopic model of molecular motors moving preferentially along biofilaments and showed by coarse-graining this model that the equation of motion for the density of filaments contains precisely the term in (*5*) responsible for curvature-induced currents.

In our experiments, we found anisotropy at the most microscopic level of single particle mo-

tion, even at time scales shorter than the vibration frequency, f. In equilibrium, the mean kinetic energies associated with the two in-plane translation degrees of freedom of the particle are equal, by the equipartition theorem, even if the particle shape is anisotropic. Figure 3 is a histogram of the magnitude of particle displacements over a time corresponding to the camera frame rate [1/300 s, or $(2/3)f^{-1}$]. The displacement along and perpendicular to the axis of the rod are displayed separately, showing that a particle is about 2.3 times as likely to move along its length as it is to move transverse to its length. Because the period of the imposed vibration (f^{-1}) sets the scale for the mean free time of the particles, this shows that the motion of the rods is anisotropic even at time scales less than or comparable to the mean free time between collisions.

We have thus presented an experimental demonstration of giant, long-lived number fluctuations in a two-dimensional active nematic. The particles in our driven system do not communicate except by contact, have no sensing mechanisms, and are not influenced by the spatially varying pressures and incentives of a biological environment. This reinforces the view that, in living matter as well, simple, nonspecific interactions can give rise to large spatial inhomogeneity. Equally important, these effects offer a counterexample to the deeply held notion that density is a sharply defined quantity for a large system.

References and Notes

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Materials and Methods
SOM Text
Figs. S1 to S6
Movies S1 and S2

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Trench-Parallel Anisotropy Produced by Foundering of Arc Lower Crust

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Many volcanic arcs display fast seismic shear-wave velocities parallel to the strike of the trench. This pattern of anisotropy is inconsistent with simple models of corner flow in the mantle wedge. Although several models, including slab rollback, oblique subduction, and deformation of water-rich olivine, have been proposed to explain trench-parallel anisotropy, none of these mechanisms are consistent with all observations. Instead, small-scale convection driven by the foundering of dense arc lower crust provides an explanation for the trench-parallel anisotropy, even in settings with orthogonal convergence and no slab rollback.

The origin of seismic anisotropy in Earth's upper mantle is attributed to the deformation-induced alignment of olivine crystals. Anisotropy can be quantified through measurements of shear-wave splitting, in which the orientation and strength of the anisotropy are estimated by measuring the polarization direction of the fastest-propagating shear wave and the delay time between the arrivals of the fastest and slowest shear waves. The relation between anisotropy and mantle flow is seen clearly at mid-ocean ridges, where fast polarization directions are oriented parallel to the spreading direction (*1*). This observation is consistent with a model in which the olivine *a* axis aligns with the transport direction inferred for corner flow beneath a ridge (*2*, *3*).

Beneath volcanic arcs, shear-wave splitting measurements frequently show fast polarization directions parallel to the strike of the arc (*4*–*6*), which rotate to a trench-normal orientation in the back-arc (*5*, *6*). Direct comparison of delay times from teleseismic *SKS* phases (which propagate through the entire mantle) (*7*) and local *S* phases generated in the subducting slab (*8*) indicates that a substantial fraction of the trench-parallel anisotropy resides in the mantle wedge above the slab (Fig. 1). Splitting measurements from local events also show strong along-arc variability in the orientation of anisotropy, with certain regions being characterized by trench-parallel anisotropy and other regions displaying more variable fast polarization directions (Fig. 1) (*8*–*10*). In contrast, two-dimensional (2D) models of slab-driven corner flow, like those invoked to explain the pattern of anisotropy at mid-ocean ridges, predict trench-normal anisotropy in the mantle wedge at subduction zones (*11*).

Deformation experiments on olivine aggregates show that the lattice preferred orientation (LPO) changes as a function of water content, stress, and temperature (*12*, *13*). Particularly intriguing is the observation of LPO controlled by the dominance of slip on the (010)[001] slip system (i.e., the B-type system). In this regime, which is predicted to dominate at high water content and low temperature/high stress, fast polarization directions are perpendicular to the flow direction (they are trench-parallel for 2D slab-driven corner flow). However, although numerical models (*14*) predict B-type fabric in the fore-arc, it is not predicted beneath the arc or in the back-arc, where trench-parallel anisotropy is often observed.

An alternative explanation for trench-parallel anisotropy is 3D flow in the mantle wedge due to oblique convergence or slab rollback (*11*, *15*). Yet, these models do not explain trench-parallel anisotropy observed in settings with nearly orthogonal convergence and little rollback (Fig. 1) (*4*, *8*). Another potential source of 3D flow in the mantle wedge is buoyancy-driven flow resulting from the foundering of high-density mafic and ultramafic cumulates into the underlying mantle (*16*). At conditions appropriate for arc lower crust (800° to 1000°C and 1 GPa) (*17*), many crustal

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assemblages are denser than the underlying mantle (*16*, *18*, *19*). Such high-density layers can become gravitationally unstable and sink into the mantle with a characteristic along-arc spacing of 30 to 50 km on time scales of ~10^6 years (fig. S1) (*18*).

Here we show that 3D flow in the mantle wedge due to lower-crustal foundering is capable of producing trench-parallel seismic anisotropy. We used finite element models to calculate instantaneous mantle flow beneath an idealized island arc [Fig. 2A and supporting online material (SOM)]. We assumed an asthenospheric viscosity of 3×10^{18} Pa·s, which is consistent with the extrapolation of experimental data to mantle wedge conditions in the presence of water and/or melt (*20*). To promote corner flow similar to that produced with temperature-dependent and/or stress-dependent viscosity (*17*, *21*), we imposed zero coupling between the downgoing slab and the lithosphere and a shallowing of the lithosphere/asthenosphere boundary directly beneath the arc (Fig. 2A).

We analyzed instantaneous flow induced by a series of spheres 16 km in diameter, located at a depth of 50 km (~20 km below the crust/mantle transition) and with an along-arc spacing of 40 km (Fig. 2A). The size and spacing of the instabilities were based on the scaling analysis and geochemical modeling of the Talkeetna arc section presented in the SOM. The spheres were 50 kg/m^3 denser than the surrounding mantle, which is appropriate for the "missing" ultramafic cumulates from the Talkeetna section (*19*). The downwelling instabilities generated small-scale return flow with a strong arc-parallel component (Fig. 2B). Trench-parallel transport was strongest in the regions of upwelling return flow, and there was a sharp transition between arc-normal flow in the back-arc and trench-parallel flow directly beneath the arc (Fig. 2B).

As a proxy for the orientation of seismic anisotropy arising from this flow field, we calculated the maximum elongation direction of the strain-rate ellipsoid (*22*). These calculations showed regions of coherent trench-parallel shear above zones of upwelling and radial patterns of shear located over zones of downwelling (Fig. 2C). The back-arc mantle was characterized by trench-normal shear. These results are consistent with the spatial pattern of anisotropy observed in shear-wave splitting measurements on local *S* phases from many subduction zones (*8*–*10*). Furthermore, our numerical results are also consistent with preserved fabrics in residual peridotites at the base of the Talkeetna section, which record trench-parallel flow rather than B-type olivine fabric (*23*).

Our model predicts trench-normal shear in the fore-arc, where some shear-wave splitting studies show trench-parallel fast polarization directions (*6*). There are several possible explanations for this discrepancy. First, in our model, instabilities are not advected trenchward with the corner flow. Thus, their influence on flow is imposed to be greatest directly below the arc. Second, although the development of B-type fabric is inconsistent with high temperatures directly below the arc, it is more likely in the colder fore-arc mantle (*14*). Finally, high-temperature fabrics produced under the arc could migrate into the fore-arc during subduction erosion.

To estimate the range of conditions over which foundering dominates the pattern of anisotropy, we compared the horizontal shear strain rate generated by Stokes flow to that from

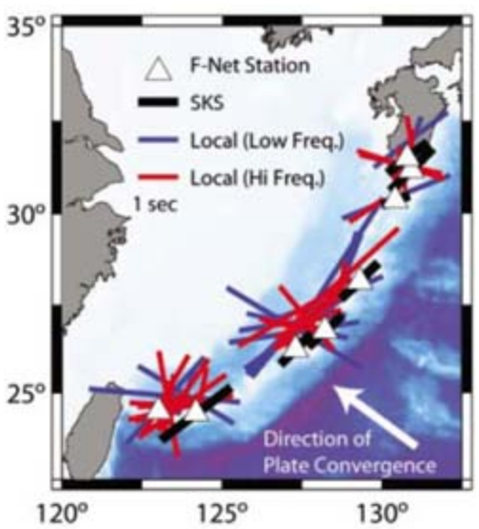

Fig. 1. Shear-wave splitting measurements from teleseismic *SKS* (thick bars) and local *S* phases (thin bars) along the Ryukyu arc (*7*, *8*). The orientation of the bars corresponds to the fast polarization direction, and bar length is scaled by delay time. Triangles denote the location of F-Net seismic stations. Splitting values for *SKS* phases are plotted at the station; values for local *S* phases are shown at the midpoint between the station and the event. Although the *SKS* measurements show relatively uniform trench-parallel fast polarization directions, the local *S* phases show a more complex pattern of anisotropy.

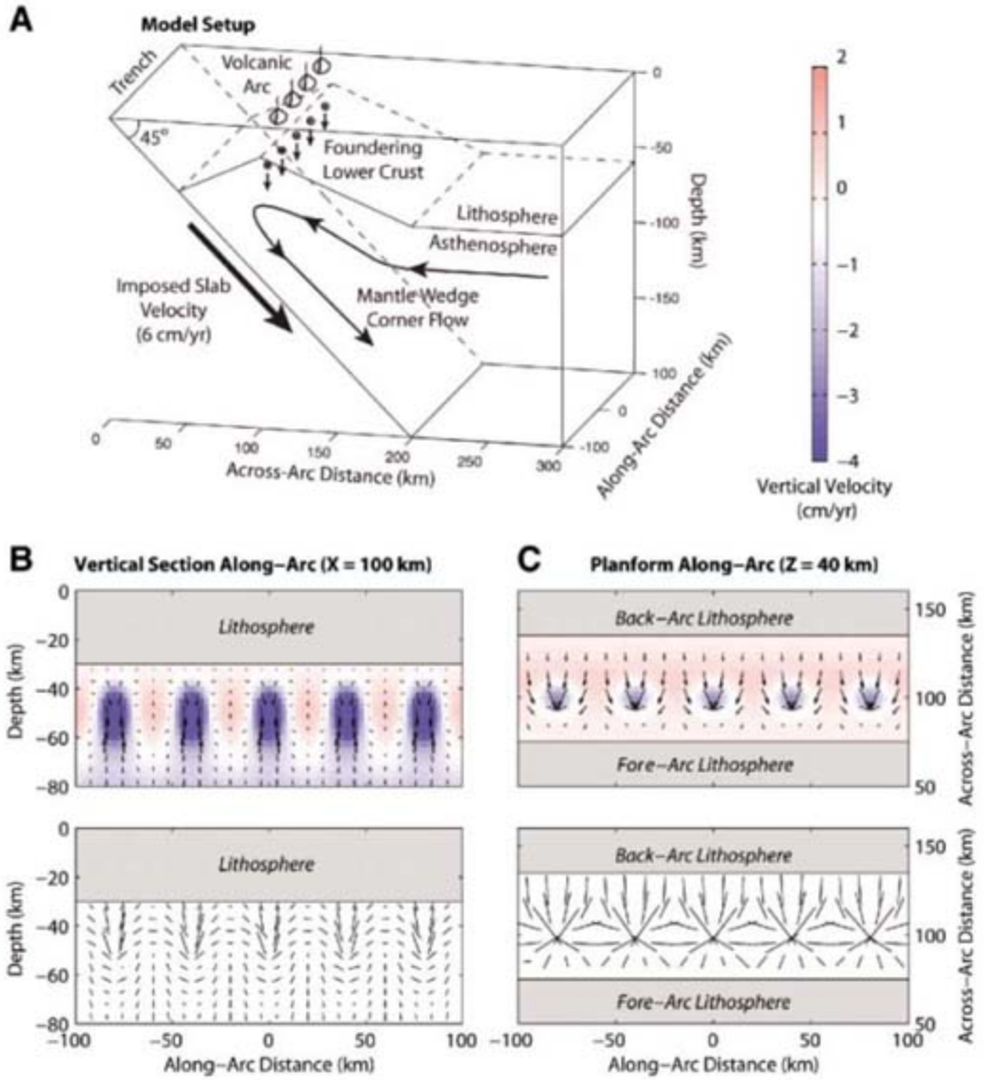

Fig. 2. (**A**) Numerical model setup for 3D calculations of mantle flow. (**B**) Vertical and (**C**) planform sections showing the orientation of the mantle velocities (top) and the long axis of the finite strain-rate ellipsoid (bottom). Foundering produces 3D flow in the mantle wedge and coherent regions of trench-parallel shear below the volcanic arc.

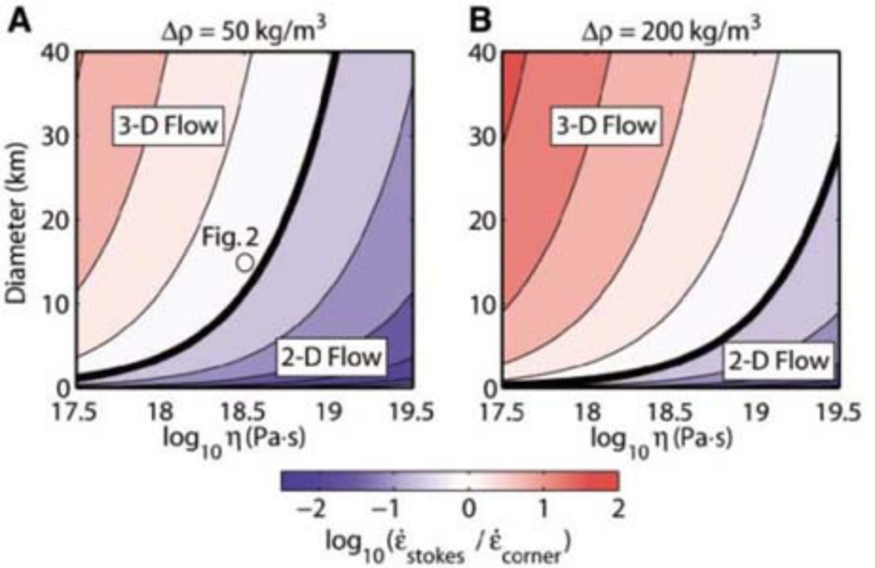

Fig. 3. Ratio of the maximum horizontal shear strain rate generated by Stokes flow, $\dot{\varepsilon}_{Stokes}$, to the mean horizontal shear strain rate associated with slab-driven corner flow, $\dot{\varepsilon}_{corner}$. Calculations are shown as a function of sphere diameter versus asthenospheric viscosity, η, assuming a slab velocity of 6 cm/year. The density contrasts relative to the surrounding mantle (Δρ) shown are appropriate for (**A**) ultramafic [(Δρ) = 50 kg/m^3] and (**B**) garnet-rich gabbroic (Δρ = 200 kg/m^3) cumulates from the Talkeetna section (*19*). For typical geologic conditions, the horizontal shear strain rates associated with Stokes flow are larger than those for corner flow, implying that lower-crustal foundering may be an important mechanism for generating 3D flow in the mantle wedge. The open circle illustrates conditions used in the 3D flow model shown in Fig. 2.

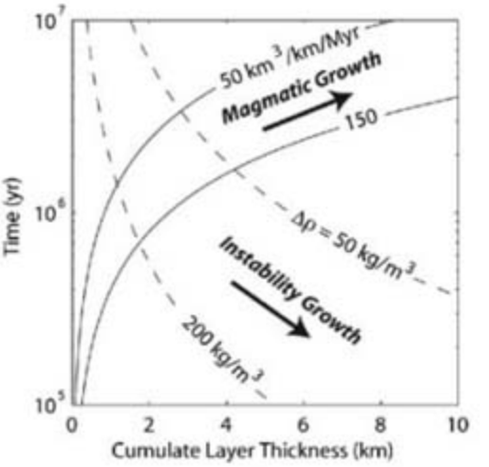

Fig. 4. Dense-layer thickness versus time, assuming magmatic fluxes of 50 to 150 km^3/km per My (solid lines). Growth rate is calculated assuming that 50% of the total magmatic flux forms a dense layer at the base of the crust. Dashed lines show instability time calculated after (*18*) as a function of cumulate layer thickness, assuming a 800°C Moho temperature for density contrasts of 50 and 200 kg/m^3 (fig. S1). For a density contrast appropriate for the missing ultramafic cumulates in the Talkeetna section (Δρ = 50 kg/m^3) and an arc flux between 50 and 150 km^3/km per My, the dense layer will grow to a thickness of ~2 to 4 km before becoming unstable.

slab-driven corner flow for a fluid of constant viscosity. The corner flow strain rate was calculated assuming a 30-km-thick lithosphere at a distance of 100 km from the trench, corresponding to the typical location of an island arc for a slab dip of 45°. Our calculations (Fig. 3) indicate that for a range of geologic conditions (for example, instability diameters of 10 to 20 km and wedge viscosities of 10^{18} to 10^{19} Pa·s), the horizontal shear strains will be strongly influenced by foundering (Figs. 2 and 3).

For foundering to affect the time-averaged flow field in the mantle wedge, the instability growth rate must be roughly balanced by the rate of crustal accretion. The rate of magma production for arcs is ~50 to 150 km^3/km per million years (My) (*24*). Assuming that about half of the magmatic flux forms a dense layer at the base of the crust (*25*), ~2 km would be added to a 30-km-wide arc every ~10^6 years (Fig. 4). The instability time for a dense layer 2 to 4 km thick is ~10^6 years at conditions appropriate for arc lower crust (Fig. 4) (*18*). These rates imply that the dense layer would grow to a thickness of 2 to 4 km over several million years before becoming unstable (intersection of solid and dashed curves in Fig. 4). Thus, we infer that instabilities with diameters of 16 to 20 km (corresponding to dense-layer thicknesses of 2 to 4 km) should form every 10^6 to 10^7 years.

Volcanic centers move within arcs on a time scale of ~10^6 years. The initiation of instabilities will probably occur between active volcanoes where igneous crust cools to <850°C near the Moho (*18*). Instability growth in these locations will in turn drive upwelling in the intervening regions. Such upwelling has the potential to induce pressure-release melting, which would in turn generate additional volcanism. Assuming 1% melt per kilobar of decompression, removing a 1- to 2-km cumulate layer over the source region for the instability will produce a minimum of ~10 km^3 of melt per instability. This process could persist through time, resulting in alternating periods of magmatic activity at arc volcanoes. An analogous process that could produce similar horizontal strains in the mantle wedge and localization of arc volcanism is upward transport of low-density diapirs composed of subducted sediment, serpentinite, and/or partial melt rising from the slab (*26*, *27*). In conclusion, the effects of 3D mantle flow induced by foundering can produce coherent regions of trench-parallel anisotropy, as well as the more complicated spatial patterns of anisotropy observed at arcs where data of higher-density shear-wave splitting data are available (*8*–*10*). Furthermore, our analysis suggests that using splitting data solely at island stations biases the interpretation of the flow field in the mantle wedge beneath the arc.

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Ancient Biomolecules from Deep Ice Cores Reveal a Forested Southern Greenland

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It is difficult to obtain fossil data from the 10% of Earth's terrestrial surface that is covered by thick glaciers and ice sheets, and hence, knowledge of the paleoenvironments of these regions has remained limited. We show that DNA and amino acids from buried organisms can be recovered from the basal sections of deep ice cores, enabling reconstructions of past flora and fauna. We show that high-altitude southern Greenland, currently lying below more than 2 kilometers of ice, was inhabited by a diverse array of conifer trees and insects within the past million years. The results provide direct evidence in support of a forested southern Greenland and suggest that many deep ice cores may contain genetic records of paleoenvironments in their basal sections.

The environmental histories of high-latitude regions such as Greenland and Antarctica are poorly understood because much of the fossil evidence is hidden below kilometer-thick ice sheets (*1*–*3*). We test the idea that the basal sections of deep ice cores can act as archives for ancient biomolecules.

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The samples studied come from the basal impurity-rich (silty) ice sections of the 2-km-long Dye 3 core from south-central Greenland (*4*), the 3-km-long Greenland Ice Core Project (GRIP) core from the summit of the Greenland ice sheet (*5*), and the Late Holocene John Evans Glacier on Ellesmere Island, Nunavut, northern Canada (Fig. 1). The last-mentioned sample was included as a control to test for potential exotic DNA because the glacier has recently overridden a land surface with a known vegetation cover (*6*). As an additional test for long-distance atmospheric dispersal of DNA, we included five control samples of debris-free Holocene and Pleistocene ice taken just above the basal silty samples from the Dye 3 and GRIP ice cores (Fig. 1B). Finally, our analyses included sediment samples from the Kap København Formation from the northernmost part of Greenland, dated to 2.4 million years before the present (Ma yr B.P.) (*1*, *2*).

The silty ice yielded only a few pollen grains and no macrofossils (*7*). However, the Dye 3 and John Evans Glacier silty ice samples showed low levels of amino acid racemization (Fig. 1A, inset), indicating good organic matter preservation (*8*). Therefore, after previous success with permafrost and cave sediments (*9*–*11*), we attempted to amplify ancient DNA from the ice. This was done following strict criteria to secure authenticity (*12*–*14*), including covering the surface of the frozen cores with plasmid DNA to control for potential contamination that may have entered the interior of the samples through cracks or during the sampling procedure (*7*). Polymerase chain reaction (PCR) products of the plasmid DNA were obtained only from extracts of the outer ice scrapings but not from the interior, confirming that sample contamination had not penetrated the cores.

Using PCR, we could reproducibly amplify short amplicons [59 to 120 base pairs (bp)] of the chloroplast DNA (cpDNA) *rbc*L gene and *trn*L intron from ~50 g of the interior ice melts from the Dye 3 and the John Evans Glacier silty samples. From Dye 3, we also obtained 97-bp amplicons of invertebrate cytochrome oxidase subunit I (COI) mitochondrial DNA (mtDNA). Attempts to reproducibly amplify DNA from the GRIP silty ice and from the Kap København Formation sediments were not successful. These results are consistent with the amino acid racemization data demonstrating superior preservation of biomolecules in the Dye 3 and John Evans Glacier silty samples, which is likely because these samples are colder (Dye 3) or younger (John Evans Glacier) than the GRIP sample (Fig. 1A, inset). We also failed to amplify DNA from the five control samples of Holocene and Pleistocene ice taken just above the silty samples from the Dye 3 and GRIP ice cores (volumes: 100 g to 4 kg; Fig. 1B) (*7*). None of the samples studied yielded putative sequences of vertebrate mtDNA.

A previous study has shown that simple comparisons of short DNA sequences to GenBank sequences by means of the Basic Local Alignment Search Tool (BLAST) make misidentification likely (*15*). Therefore, we assigned the obtained sequences to the taxonomic levels of order, family, or genus using a new rigorous statistical approach (*7*). In brief, this Bayesian method calculates the probability that each sequence belongs to a particular clade by considering its position in a phylogenetic tree based on similar GenBank sequences. In the calculation of these probabilities, uncertainties regarding phylogeny, models of evolution, and missing data are taken into account. Sequences with >90% posterior probability of membership to a taxonomic group were assigned to that group. Additionally, a given plant taxon was only considered genuine if sequences assigned to that taxon were found to be reproducibly obtained in separate analyses (by independent laboratories for the Dye 3 sample and within the laboratory for the John Evans Glacier control sample). This strict

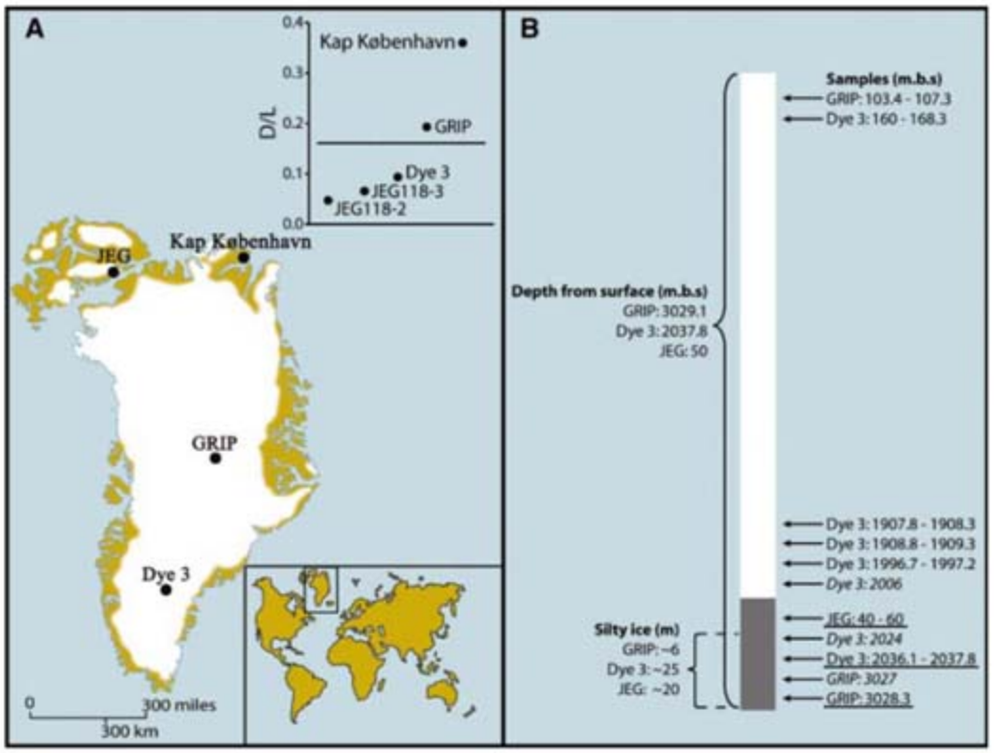

Fig. 1. Sample location and core schematics. (**A**) Map showing the locations of the Dye 3 (65°11'N, 45°50'W) and GRIP (72°34'N, 37°37'W) drilling sites and the Kap København Formation (82°22'N, W21°14'W) in Greenland as well as the John Evans Glacier (JEG) (79°49'N, 74°30'W) on Ellesmere Island (Canada). The inset shows the ratio of D– to L–aspartic acid, a measure of the extent of protein degradation; more highly degraded samples (above the line) failed to yield amplifiable DNA. (**B**) Schematic drawing of ice core/icecap cross section, with depth [recorded in meters below the surface (m.b.s.)] indicating the depth of the cores and the positions of the Dye 3, GRIP, and JEG samples analyzed for DNA, DNA/amino acid racemization/luminescence (underlined), and $^{10}Be/^{36}Cl$ (italic). The control GRIP samples are not shown. The lengths (in meters) of the silty sections are also shown.

criterion of authenticity obviously dismisses many putative taxa that are present at low abundance or have heterogeneous distributions, as is typical of environmental samples (*16*), but efficiently minimizes the influence of possible low-level contamination and misidentifications due to DNA damage (*17*).

Approximately 31% of the sequences from the John Evans Glacier silty sample were assigned to plant taxa that passed the authentication and identification criteria. These belong to the order Rosales, the family Salicaceae, and the genus *Saxifraga* (Table 1). This result is consistent with the John Evans Glacier forming no more than a few thousand years ago in a high Arctic environment (*18*), characterized by low plant diversity and sparse vegetation cover similar to that currently surrounding the glacier, which consists mainly of Arctic willow (Salicaceae), purple saxifrage (*Saxifraga*), *Dryas* (Rosales), and Arctic poppy (*Papaver*) (*19*). Thus, by confirming the expected result, the John Evans Glacier study can be regarded as a positive control, showing that DNA data from silty ice reliably record the local ecology.

In contrast to the John Evans Glacier silty sample, the 45% of the Dye 3 DNA sequences that could be assigned to taxa reveal a community very different from that of Greenland today. The taxa identified include trees such as alder (*Alnus*), spruce (*Picea*), pine (*Pinus*), and members of the yew family (Taxaceae) (Table 1). Their presence indicates a northern boreal forest ecosystem rather than today's Arctic environment. The other groups identified, including Asteraceae, Fabaceae, and Poaceae, are mainly

Table 1. Plant and insect taxa obtained from the JEG and Dye 3 silty ice samples. For each taxon (assigned to order, family, or genus level), the genetic markers (*rbc*L, *trn*L, or COI), the number of clone sequences supporting the identification, and the probability support (in percentage) are shown. Sequences have been deposited in GenBank under accession numbers EF588917 to EF588969, except for seven sequences less than 50 bp in size that are shown below. Their taxon identifications are indicated by symbols.

Order	Marker	Clones	Support (%)	Family	Marker	Clones	Support (%)	Genus	Marker	Clones	Support (%)
JEG sample											
Rosales	*rbc*L	3	90–99								
Malpighiales	*rbc*L	2	99–100	Salicaceae	*rbc*L	2	99–100				
	*trn*L	5	99–100		*trn*L	4	100				
Saxifragales	*rbc*L	3	92–94	Saxifragaceae	*rbc*L	2	92	*Saxifraga*	*rbc*L	2	91
Dye 3 sample											
Coniferales	*rbc*L	44	97–100	Pinaceae*	*rbc*L	20	100	*Picea*	*rbc*L	20	99–100
	*trn*L	27	100		*trn*L	25	100	*Pinus*†	*trn*L	17	90–99
				Taxaceae‡	*rbc*L	23	91–98				
					*trn*L	2	100				
Poales§	*rbc*L	67	99–100	Poaceae§	*rbc*L	67	99–100				
	*trn*L	17	97–100		*trn*L	13	100				
Asterales	*rbc*L	18	90–100	Asteraceae	*rbc*L	2	91				
	*trn*L	27	100		*trn*L	27	100				
Fabales	*rbc*L	10	99–100	Fabaceae	*rbc*L	10	99–100				
	*trn*L	3	99		*trn*L	3	99				
Fagales	*rbc*L	10	95–99	Betulaceae	*rbc*L	8	93–97	*Alnus*	*rbc*L	7	91–95
	*trn*L	12	100		*trn*L	11	98–100		*trn*L	9	98–100
Lepidoptera	COI	12	97–99								

*Env_2, *trn*L ATCCGGTTCATGAAGACAATGTTTCTTCTCCTAAGATAGGAAGGG. Env_3, *trn*L ATCCGGTTCATGAAGACAATGTTTCTTCTCCTAATATAGGAAGGG. Env_4, *trn*L ATCCGGTTCATGAGGACAATGTTTCTTCTCCTAATATAGGAAGGG. †Env_5, *trn*L CCCTTCCTATCTTAGGAGAAGAAACATTGTCTTCATGAACCGGAT. Env_6, *trn*L TTTCCTATCTTAGGAGAAGAAACATTGTCTTCATGAACCGGAT. ‡Env_1, *trn*L ATCCGTATTATAGGAACAATAATTTTATTTTCTAGAAAAGG. §Env_7, *trn*L CTTTTCCTTTGTATTCTAGTTCGAGAATCCCTTCTCAAAACACGGAT.

herbaceous plants and are represented by many species found in northern regions at present (Table 1). The presence of these herb-dominated families suggests an open forest where heliophytes thrived. Additionally, we recorded taxa that are common in present-day Arctic and/or boreal regions but lacked identity between independent laboratories. These are yarrow (*Achillea*), birch (*Betula*), chickweed (*Cerastium*), fescue (*Festuca*), rush (*Luzula*), plantain (*Plantago*), bluegrass (*Poa*), saxifrage (*Saxifraga*), snowberry (*Symphoricarpos*), and aspen (*Populus*). Although not independently authenticated at the sequence level, the presence of these taxa adds further support to the former existence of a northern boreal forest ecosystem at Dye 3.

To date, the youngest well-dated fossil evidence of native forest in Greenland is from macrofossils in the deposits of the Kap København Formation from the northernmost part of Greenland and dates back to around 2.4 Ma (*1*, *2*). Other less well dated traces of forests in Greenland include wood at two other late Cenozoic sites in northern Greenland (*20*), pollen spectra of unknown age in marl concretions found in a late glacial moraine, and wood and spruce seeds in eastern Greenland (*21*). The core from Dye 3, located almost exactly 2000 km to the southwest of the Kap København Formation (Fig. 1A), therefore, provides direct evidence of a forested southern-central Greenland.

The invertebrate sequences obtained from the Dye 3 silty ice are related to beetles (Coleoptera), flies (Diptera), spiders (Arachnida), brushfoots (Nymphalidae), and other butterflies and moths (Lepidoptera) (taxonomic identification probability support between 50 and 99%). However, only sequences of the Lepidoptera are supported by more than 90% significance (Table 1). Thus, although detailed identifications of the COI sequences are in general not strongly supported, the results show that DNA from a variety of invertebrates can be obtained from sediments even in the absence of macrofossils, as was previously shown for plants, mammals, and birds (*9*–*11*).

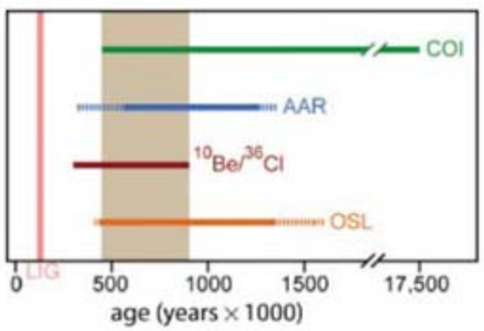

Fig. 2. Summary of dating results for the silty ice from Dye 3. From top to bottom, the bars indicate: maximum likelihood estimates for the branch length of the invertebrate COI sequences (COI); amino acid racemization results with the use of alternative activation energies, models of racemization behavior, and basal temperature histories (AAR); age estimate from ^{10}Be/^{36}Cl measurements in silty ice; and minimum ages based on single-grain luminescence results (optically stimulated luminescence or OSL). The time span covered by all dating methods (450 to 800 ka) is marked in gray. Stippled lines represent the results of less likely models. The maximum age estimate for the invertebrate COI sequences is based on an unlikely slow substitution rate [for details, see text and (*6*)].

Several observations suggest that the DNA sequences we obtained from the Dye 3 ice are of local origin and not the result of long-distance dispersal. The reproducible retrieval of diverse DNA from the silty basal ice but not from similar or larger volumes of the overlying "clean" ice largely precludes long-distance atmospheric dispersal of microfossils as a source of the DNA.

Although pollen grains are found in the Greenland ice sheet, including the Dye 3 silty ice (*7*), the concentrations are in general too low [~15 grains per liter (*22*, *23*)] for them to be present in the sample volumes studied. Furthermore, long-term survival of DNA in pollen has proved fairly poor (*24*), and the vast majority of angiosperm pollen does not contain cpDNA (*25*). These factors effectively exclude pollen as the general source of plant DNA from the silty ice. Moreover, the Dye 3 silty ice appears to have originated as solid precipitation without going through stages of superimposed ice and most likely formed by mixing in the absence of free water (i.e., ice that has never melted) (*26*), effectively excluding subsurface transportation. As explained in (*27*), the ice is believed to be predominantly of local origin, having been shielded from participating in the large-scale glacier flow by a bedrock trough, in agreement with the solid ice–mixing hypothesis (*26*). Thus, being of local origin, the DNA sequences from the Dye 3 silty ice must be derived from the plants and animals that inhabited this region the last time that it was ice-free, because possible older DNA records from previous ice-free periods will vanish with the establishment of a new ecosystem, or at least be out-competed during PCR by DNA from the most recent record. The plant taxa suggest that this period had average July temperatures that exceeded 10°C and winter temperatures not colder than –17°C, which are the limits for northern boreal forest and *Taxus*, respectively (*1*). Allowing for full recovery of the isostatic depression that is produced by 2 km of ice, Dye 3 would have been ~1 km above sea level. In combination, these factors suggest that a high-altitude boreal forest at Dye 3 may date back to a period considerably warmer than present.

There are no established methods for dating basal ice, and it remains uncertain whether the overlying clean ice of Dye 3 is temporally contiguous with the lower silty section. Therefore, to obtain a tentative age estimate for the Dye 3 silty ice and its forest community, we applied a series of dating techniques: ^{10}Be/^{36}Cl isotope ratios, single-grain luminescence measurements, amino acid racemization coupled with modeling of the basal ice temperature histories of GRIP and Dye 3, and maximum likelihood estimates for the branch length of the invertebrate COI sequences (*7*). All four dating methods suggest that the Dye 3 silty ice and its forest community predate the Last Interglacial (LIG) [~130 to 116 thousand years ago (ka)] (Fig. 2), which contrasts with the results of recent models suggesting that Dye 3 was ice-free during this period (*28*, *29*). Indeed, all four dating methods give overlapping dates for the silty ice between 450 and 800 ka (Fig. 2), exceeding the current record of long-term DNA survival from Siberian permafrost of 300 to 400 ka (*9*). However, because of the many assumptions and uncertainties connected with the interpretation of the age estimates (*7*), we cannot rule out the possibility of a LIG age for the Dye 3 basal ice.

Our results reveal that ancient biomolecules from basal ice offer a means for environmental reconstruction from ice-covered areas and can yield insights into the climate and the ecology of communities from the distant past. Because many deep ice cores exist from both hemispheres and further drillings are planned, this approach may be used on a larger scale. Basal ice at even lower temperatures than Dye 3 might contain an archive of genetic data of even greater antiquity.

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Figs. S1 to S8
Tables S1 to S8
References

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Middle Paleolithic Assemblages from the Indian Subcontinent Before and After the Toba Super-Eruption

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The Youngest Toba Tuff (YTT) eruption, which occurred in Indonesia 74,000 years ago, is one of Earth's largest known volcanic events. The effect of the YTT eruption on existing populations of humans, and accordingly on the course of human evolution, is debated. Here we associate the YTT with archaeological assemblages at Jwalapuram, in the Jurreru River valley of southern India. Broad continuity of Middle Paleolithic technology across the YTT event suggests that hominins persisted regionally across this major eruptive event.

The Youngest Toba Tuff (YTT) eruption of 74,000 years ago (74 ka) was Earth's largest volcanic event in the past two million years (*1*–*3*). It was two orders of magnitude larger (in erupted mass) than the largest known historic eruption, that of Tambora, also in Indonesia (*4*). The YTT involved the eruption of a minimum of 2800 km^3 (7×10^{15} kg) of magma, of which at least ~800 km^3 was transported in atmospheric ash plumes that blanketed an area from the South China Sea to the Arabian Sea (*2*, *3*). Its impact on Earth's atmosphere and climate (*5*–*7*) and on local animal and plant populations remains a matter of contention (*5*, *7*–*12*).

The Indian subcontinent contains extensive YTT deposits (*13*–*15*). Here we describe an archaeological sequence from south India that includes a substantial YTT layer and sheds light on the eruption's impact on climate, environments, and hominin populations. In the Kurnool District of Andhra Pradesh in southern India, stratified archaeological sites in the Jurreru River valley contain stone artifacts in association with faunal remains in caves, rockshelters, and open-air localities (*16*, *17*) (Fig. 1). The archaeological record spans all periods of the Paleolithic. In addition, current mining activities have exposed tephra deposits over an area of 64 ha. Ash is, however, certainly buried over a wider area within the valley (fig. S1), and we estimate its total volume at $7 \pm 0.7 \times 10^5$ m^3, based on the interpolation of 225 depth observations made at mining exposures.

We conducted electron probe microanalysis (EPMA) of volcanic glass shards from the Jwalapuram tephra to compare their geochemical signatures with those of the Older Toba Tuff (OTT, dated to ~840 ka) and the Middle Toba Tuff (MTT, dated to ~500 ka) (*4*). The results show that the Jwalapuram ash is a distal deposit of the YTT (figs. S3 and S4), based on its close similarities with proximal deposits of YTT in Sumatra and with previously characterized distal occurrences in India (*13*, *14*, *18*).

Jwalapuram locality 3 preserves more than 7.5 m of sedimentary deposits, including a 2.55-m-thick deposit of ash, and a sequence of lithic artifacts that straddle the ash layer (fig. S2). Soft sediment deformation structures suggest that the tephra initially accumulated on a wet clay substrate, probably in a lacustrine environment. The abrupt transition from light gray ash to an orange (but still ash-rich) silt horizon immediately above the ash sequence represents a major change in depositional regime. We interpret this as evidence that the lake dried up soon after the ash fall, possibly during the onset of glacial conditions in oxygen isotope stage 4.

The stone tool assemblages were found in trenches placed across the landscape (that is, at Jwalapuram localities 3, 17, and 21). At Jwalapuram locality 3, we used optical dating to obtain burial ages for sediment samples from archaeological layers above (JLP-380) and below (JLP3A-200) the ash. Ages of 77 ± 6 and 74 ±

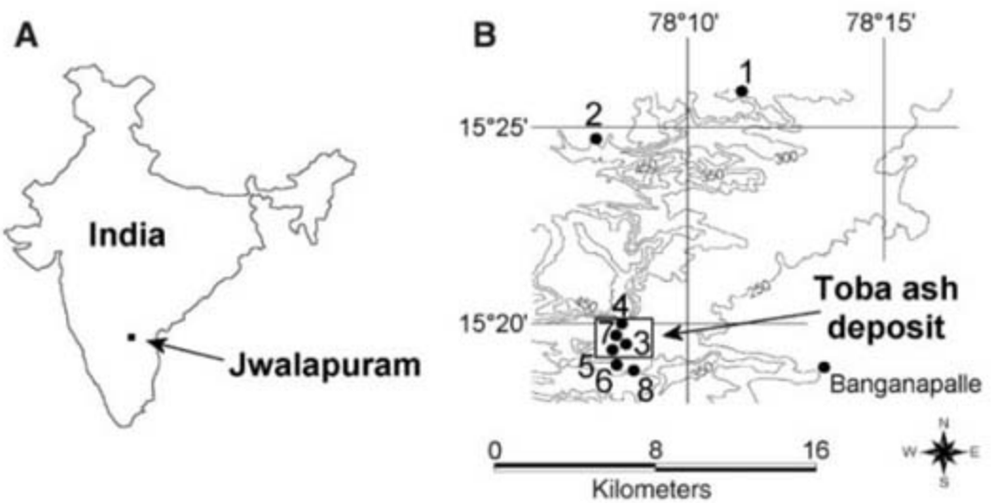

Fig. 1. Location of Jwalapuram, archaeological sites, and tephra deposits. (**A**) Location of the Jwalapuram study area. (**B**) Key archaeological localities in the Kurnool District include the Upper Paleolithic caves of Billasurgum (1) (*17*) and Muchchatla Chintamanu Gavi (2) (*16*). Jwalapuram localities include 17 (3, Middle Paleolithic), 9 (4, Microlithic), 3 (5, Middle Paleolithic), 20 (6, Middle Paleolithic), 21 (7, Middle Paleolithic), and Tank (8, Acheulean).

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7 ka were obtained for the pre- and post-Toba samples, respectively (tables S2 and S3). These indicate that the dated quartz grains were last exposed to sunlight shortly before and after the Toba eruption, with no substantial hiatus in sediment deposition.

The pre-Toba archaeological layer at locality 3, chronologically bracketed by the ~74,000-year-old YTT and the underlying sediments dated to 77 ± 6 ka, contained 215 artifacts as well as a piece of red ochre that shows striations due to use. This stone tool assemblage consists of faceted unidirectional cores made from limestone (60%), quartzite (22%), and chert (11%), with elongate parallel flake scars indicating the production of blades. Frequent preparation of flake platforms is seen, suggesting that these flakes were struck from prepared cores similar to those found at the site. A small proportion of flakes were retouched into notches, informal scrapers, retouched blades, and a burin (Fig. 2). This pre-Toba assemblage falls within the Indian Middle Paleolithic (*19*, *20*).

The post-Toba layer at locality 3, optically dated to 74 ± 7 ka, contains an assemblage of 108 stone artifacts that occur throughout the orange sandy stratum; a further 37 and 131 artifacts were recovered from the same matrix above the ash at localities 17 and 21, respectively. The technology and tool types at these three post-ash localities are similar to those found in the pre-ash assemblage, involving faceted unidirectional cores with some blade scars (Fig. 2). However, raw materials were used in different frequencies (limestone 31%, chert 28%, chalcedony 23%, and quartzite 12%). Most flakes are short and squat, although a few blades and bladelets (<2 cm in length) are also present (<5%), along with a bladelike core and a small bidirectional blade core with a faceted platform (Fig. 2). Retouched flakes above the ash include notches and side and end scrapers. Burins and bipolar reduction are also present, but rare. This combination of tool types is common in Late Pleistocene assemblages of India, usually identified as Middle Paleolithic (*19*, *20*).

We provide here firm chronological evidence that hominins were present in the Jurreru River valley, south India, immediately before and after the YTT eruption. Analyses of the archaeological industries recovered from the site indicate a strong element of technological continuity between the pre- and post-Toba assemblages. Together with the presence of faceted unidirectional and bidirectional bladelike core technology, these pre- and post-Toba industries suggest closer affinities to African Middle Stone Age traditions (such as Howieson's Poort) than to contemporaneous Eurasian Middle Paleolithic ones that are typically based on discoidal and Levallois techniques (Fig. 3). The coincidence of (i) evidence of hominins flexible enough to exhibit continuity through a major eruptive event, (ii) technology more similar to the Middle Stone Age than the Middle Paleolithic, and (iii) overlap of the Jwalapuram artifact ages with the

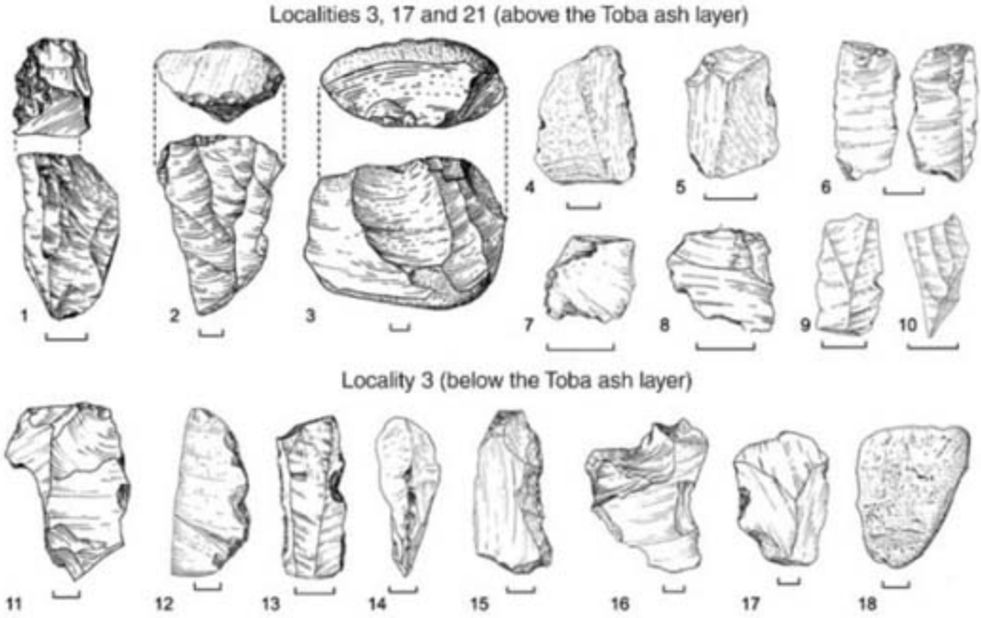

Fig. 2. Selected Jwalapuram artifacts that pre-date (locality 3) and post-date (localities 3, 17, and 21) the YTT. Above the ash: 1, bladelet core with faceted platform; 2 and 3, flake cores with faceted platforms; 4, side scraper; 5, utilized flake; 6, atypical end scraper on blade; 7, side and end scraper; 8, utilized flake; 9, broken blade; 10, broken blade. Below the ash: 11, notch and burin; 12, ventrally retouched side scraper; 13, side scraper on broken blade; 14, side scraper on ridge straightening flake; 15, ventrally retouched side and end scraper; 16, ventrally retouched scraper; 17, notch; 18, ground ochre. Scale bar, 1 cm.

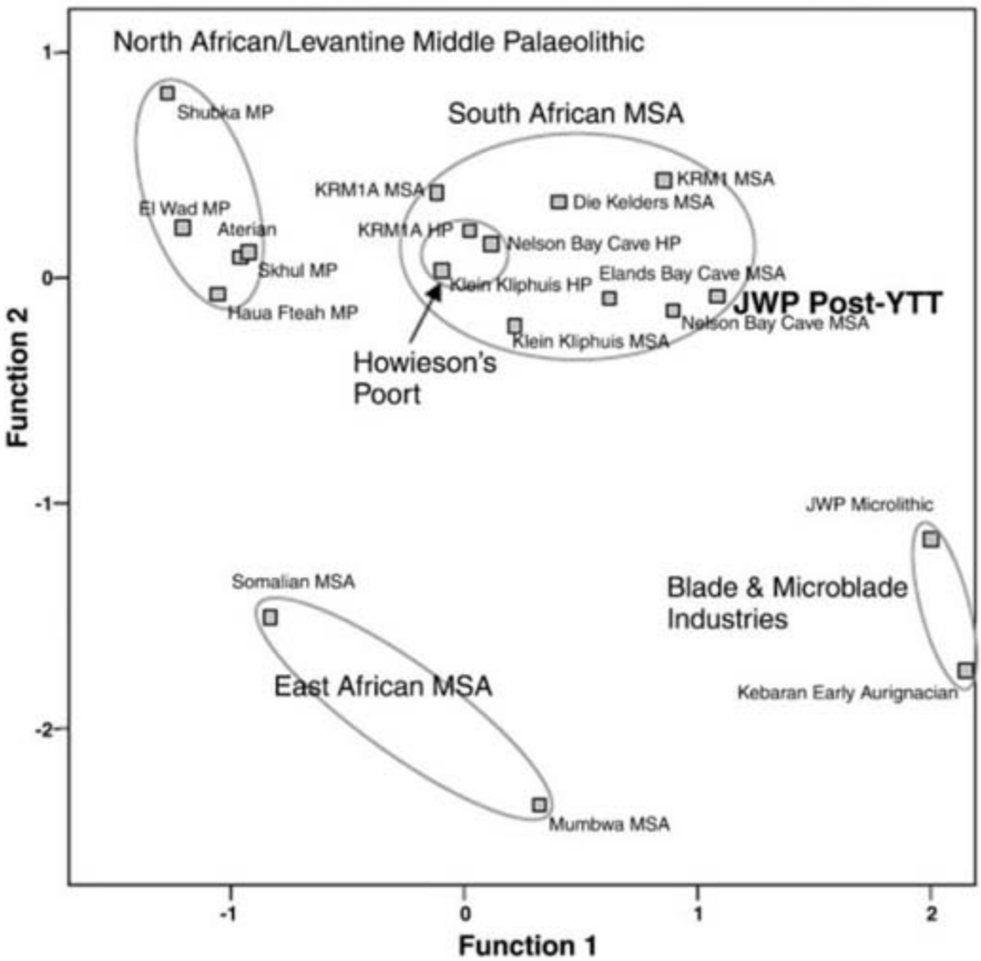

Fig. 3. Discriminant analysis of 670 cores from Middle Stone Age (MSA), Middle Paleolithic (MP), and early Upper Paleolithic (UP) contexts in Africa, the Levant, and India. Functions 1 and 2 account for 70.1% of the variation. Functions 1 to 3 are all significant at the $P = <0.0005$ level. JWP, Jwalapuram; KRM, Klasies River Mouth.

earlier end of the most commonly cited genetic coalescence dates (*21*–*23*) may suggest the presence of modern humans in India at the time of the YTT event. This interpretation would be consistent with a southern route of dispersal of modern humans from the Horn of Africa (*24*); the latter, however, will remain speculative until other Middle Paleolithic sites in the Indian subcontinent and Arabian Peninsula (*25*) are excavated and dated.

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SOM Text
Figs. S1 to S14
Tables S1 to S6
References

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Buddenbrockia Is a Cnidarian Worm

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A major evolutionary divide occurs in the animal kingdom between the so-called radially symmetric animals, which includes the cnidarians, and the bilaterally symmetric animals, which includes all worm phyla. *Buddenbrockia plumatellae* is an active, muscular, parasitic worm that belongs to the phylum Myxozoa, a group of morphologically simplified microscopic endoparasites that has proved difficult to place phylogenetically. Phylogenetic analyses of multiple protein-coding genes demonstrate that *Buddenbrockia* is a cnidarian. This active muscular worm increases the known diversity in cnidarian body plans and demonstrates that a muscular, wormlike form can evolve in the absence of overt bilateral symmetry.

Most metazoans (true animals), including arthropods, annelids, mollusks, chordates, and all worm phyla, belong to the Bilateria. This clade excludes cnidarians, ctenophores, sponges, and placozoans. Myxozoans were originally placed outside the Metazoa, despite the presence of characters such as multicellularity of spores, septate junctions, and putative nematocysts (*1*–*3*). Sequencing of 18*S* ribosomal DNA (rDNA) confirmed that they are highly modified metazoans (*4*). However, precisely placing them in the animal kingdom has proven difficult. Most myxozoans are microscopic aquatic endoparasites with either plasmodial or sac-shaped bodies, with no gross similarity to other animals. There are two classes of myxozoans, the clades Myxosporea, with over 2000 species, and the Malacosporea, with two described species and two others recently identified by rDNA comparisons (*5*). Myxozoans parasitize a wide range of hosts, including fish, annelids, and (for malacosporeans) bryozoans. Myxozoans form complex spores containing polar capsules similar to the stinging organelles (nematocysts) of cnidarians, which they use to attach to a new host. Polar capsules differ from typical nematocysts of cnidarians in lacking chemo- and/or mechanosensory structures and neural connections that modulate discharge (*6*).

If polar capsules and nematocysts are homologous, then myxozoans could be cnidarians or the sister group to cnidarians. Alternatively, nematocyst-like structures may have evolved before the divergence of cnidarians and bilaterians, or they could have arisen independently. Some analyses of myxozoan 18*S* rDNA sequences have also suggested that myxozoans are related to cnidarians, most notably, when the highly divergent rDNA sequence of the endoparasitic cnidarian *Polypodium hydriforme* is included (*3*). In contrast, other rDNA analyses suggest myxozoans are bilaterians (*7*, *8*). These contradictory phylogenetic results may be a consequence of the highly divergent (long-branch) rDNA sequences of myxozoans (*9*), making placement difficult.

The report of bilaterian-like Hox genes in two myxozoan species (*10*) and the surprising finding that a rare endoparasitic worm that infects freshwater bryozoans, *Buddenbrockia plumatellae* (*11*) (Fig. 1), is actually a myxozoan (*7*, *12*) have further confounded the placement of the myxozoans. *Buddenbrockia* worms are highly active, with continuous and vigorous sinuous writhing within the body cavity of bryozoan hosts (*12*, *13*). The worms escape from their bryozoan hosts, probably through the vestibular pore, and undergo repeated coiling and straightening (*13*). The vermiform (wormlike) body plan of *Buddenbrockia* is reminiscent of bilaterian taxa, although *Buddenbrockia* lacks a recognizable nervous system, gut, and external

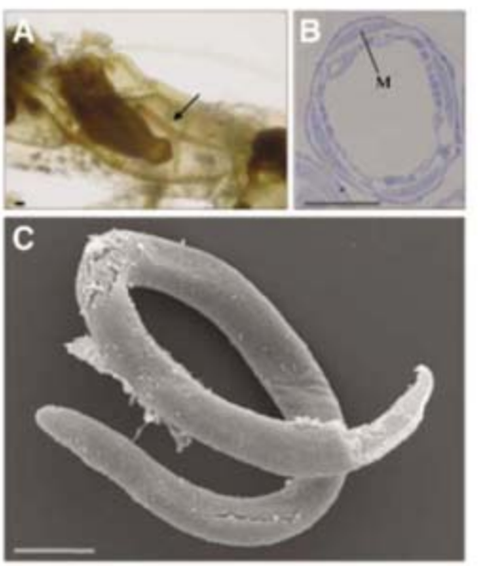

Fig. 1. (**A**) A zooid of the bryozoan *Plumatella* with *Buddenbrockia* worms (arrow) in the body cavity. Scale bar, 40 μm. (**B**) Cross section of an immature *Buddenbrockia plumatellae* worm. Note the presence of four longitudinal muscle blocks (M) and absence of gut. Scale bar, 20 μm. (**C**) Scanning electron microscopy image of a *Buddenbrockia plumatellae* worm. Scale bar, 100 μm.

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sense organs (*9*, *11*, *12*) (Fig. 1). The sinuous movements of the body are bilaterian-like and quite unlike those of elongate cnidarians. For instance, planula larvae creep along the substratum, burrowing cerianthids are only capable of retraction, burrowing anemones undergo peristalsis (*14*), and the swimming narcomedusan *Tetraplatia* uses specialized locomotory flaps (*15*). In contrast, the four well-defined blocks of longitudinal muscles running the length of the body in *Buddenbrockia* (Fig. 1B) are more comparable to those of nematodes and nematomorphs. Although some cnidarians have four longitudinal muscles that run the length of the individual (Stauromedusae, for example), these animals are not vermiform.

Despite its gross dissimilarity to all other members of the phylum, there is strong evidence that this strange vermiform animal is indeed a true myxozoan. First, ultrastructural analyses revealed that *Buddenbrockia* has polar capsules similar to those of malacosporean myxozoans; these are found in infective spores and also in the epidermis of the worm (*12*). Additionally, both malacosporeans and the *Buddenbrockia* worm have an unusual form of cell junction in the body wall and use freshwater bryozoans as hosts (*12*). The 18*S* rDNA sequence of vermiform *Buddenbrockia plumatellae* is similar to that of the malacosporean myxozoan, *Tetracapsula bryozoides* [now revised to *B. plumatellae* (*13*)], which suggests that they are at least congeneric (*7*).

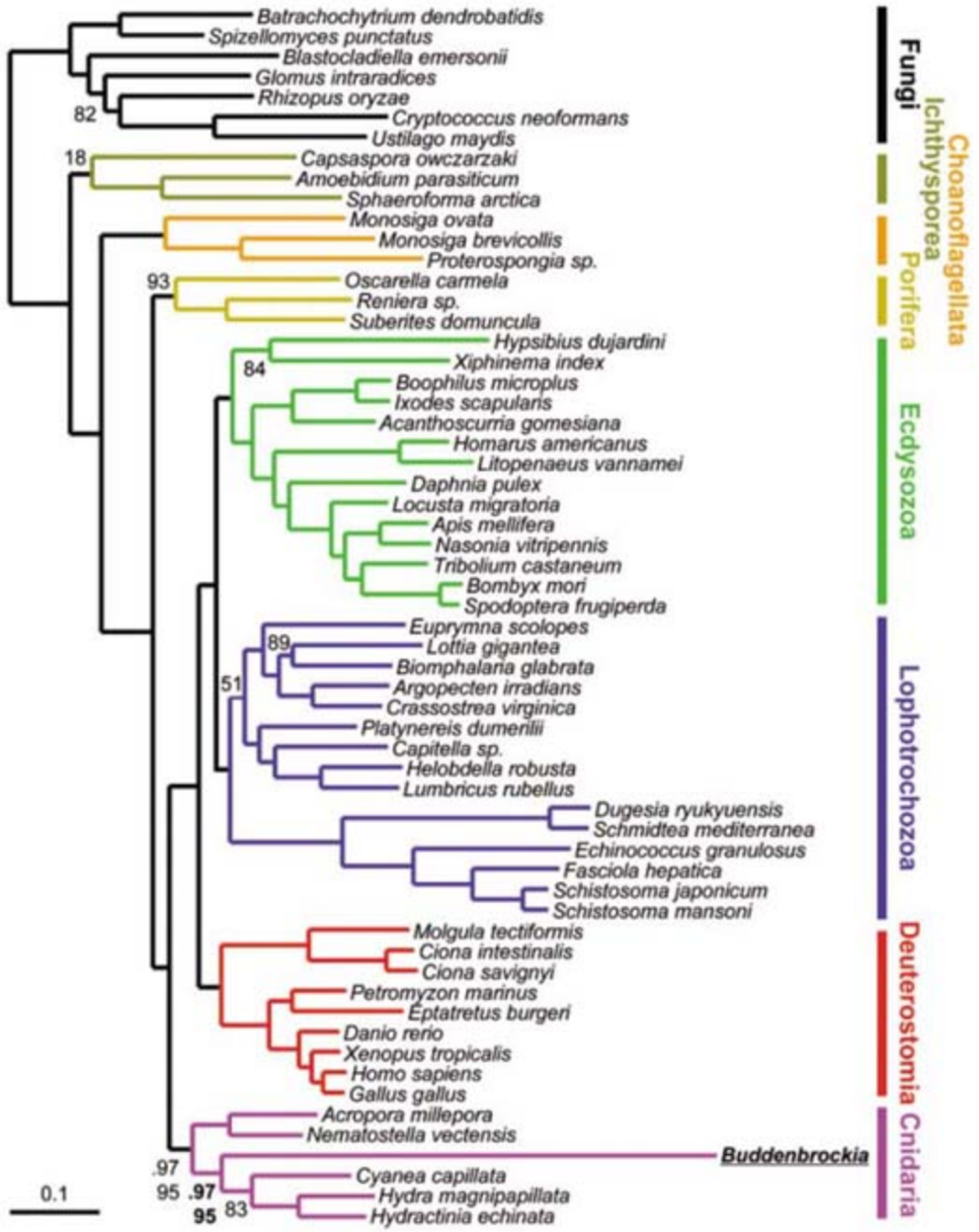

Fig. 2. Phylogenetic analyses of genomic data strongly support the grouping of *Buddenbrockia* and Medusozoa. Bayesian tree obtained from the analysis of 31,092 aligned amino acid positions. Posterior probabilities are equal to 1 except for the two nodes where 0.97 values are indicated. Bootstrap values obtained with the CAT model are indicated when <98% (see text and SOM). Scale bar indicates number of changes per site.

To investigate the phylogenetic affinities of myxozoans, we tested the veracity of the four Hox gene sequences previously reported from the myxozoans *Tetracapsula bryozoides* and *Myxidium lieberkuehni* (*10*). Surprisingly, polymerase chain reaction (PCR) with gene-specific primers amplified three of the genes *Myx1*, *Myx2*, and *Myx3*, from uninfected bryozoans (*Cristatella mucedo*). These bryozoans were collected from Littoistenjärvi, Finland, where myxozoan infection has not been observed, and were shown to be parasite-free by PCR amplification and sequencing of rDNA. The *Myx4* gene was amplified from Northern pike (*Esox lucius*), a natural host of the myxozoan *M. lieberkuehni*. All amplified sequences were verified by cloning and sequencing. These genes did not amplify in any of our myxozoan samples with the same gene-specific primers, which indicated that the Hox gene sequences reported from myxozoans derive from host DNA. We then turned to the phylogenetic analysis of orthologous nuclear protein-coding genes (*16*). To ensure that these were cloned from myxozoan tissue, and not contaminated by host tissue, we initially used universal PCR primers targeted to 18*S* rDNA (*17*) to screen a range of adult and spore samples from four species of myxozoan. Each amplified PCR band was cloned and sequenced to assess levels of contamination in each sample. A myxozoan sample free from contamination was obtained by dissecting infected bryozoan colonies and collecting individual *Buddenbrockia* worms released from the disrupted body cavity. Each worm is around 2 mm in length (range 0.05 to 3.6 mm). These samples yielded only myxozoan rDNA sequences after PCR amplification with universal primers (50/50 clones sequenced).

From a sample of 10 *Buddenbrockia* worms not contaminated by host DNA, we cloned a total of 50 different protein-coding genes that previously had been identified as unequivocal single-copy genes (*18*). Orthology was confirmed by phylogenetic analysis of each gene. We aligned 129 proteins (29,773 unambiguously aligned amino acid positions) from a wide diversity of animal species (47 animals and 13 outgroups), including *Buddenbrockia*, three sponges, five cnidarians, 14 ecdysozoans, 15 lophotrochozoans, and nine deuterostomes, choosing taxa from each group on the basis of the shortest branch lengths (tables S1 to S3, see SOM text). A Bayesian tree inferred with a WAG+Γ model (*16*) (Fig. 2) was in agreement with the current view of animal evolution (*19*). In this phylogeny, *Buddenbrockia* is placed within the Cnidaria, forming a clade with Medusozoa (Hydrozoa plus Scyphozoa), to the exclusion of Anthozoa (with a posterior probability of 0.97). To further evaluate the robustness of this result, we analyzed the 115 possible positions of *Buddenbrockia* in a backbone tree lacking *Buddenbrockia* (fig. S1). This phylogenetic placement was favored over all others. However, the bootstrap support for this node was only 70%, and five alternative posi-

tions cannot be rejected by the approximately unbiased (AU) multiscale bootstrap test (*20*) at the 5% level (fig. S2). This uncertainty may relate to the fact that *Buddenbrockia* genes have undergone rapid sequence evolution, which can either cause artifactual groupings or reduce the support for the correct grouping (*21*, *22*). This is not expected to be the cause of the grouping between *Buddenbrockia* and Medusozoa, because the branches of both the Hydrozoa and Scyphozoa species are short and should not act as a long-branch attractor. When trees were inferred by parsimony, a method highly susceptible to long-branch attraction (*23*), *Buddenbrockia* was grouped with an artifactual clade of long-branch platyhelminths and nematodes, not Medusozoa (fig. S3). To circumvent the long-branch attraction effect (*24*, *25*), we reanalyzed the data under the CAT model, which explicitly handles the heterogeneity of the substitution process across positions (*26*). The CAT tree (fig. S4) was identical to the Bayesian tree except for the relative placement of some nonmetazoan branches. It is noteworthy that less phylogenetic resolution was observed within the *Buddenbrockia* + Medusozoa clade, as these results suggest that *Buddenbrockia* is either an outgroup to Scyphozoa plus Hydrozoa (83% CAT) or sister to Hydrozoa (17% CAT). On the basis of these data, we conclude that the *Buddenbrockia* worm is a cnidarian. This conclusion can be extrapolated to all Myxozoa, because previous work has established that *Buddenbrockia* is a member of this clade (*7*, *9*). Therefore, the taxon Myxozoa should be placed within the phylum Cnidaria, on the medusozoan lineage.

Our data also show that, not only has anatomical simplification occurred in myxozoan evolution, but so has evolution of a muscular vermiform body. We infer that active, motile worms are not restricted to the bilaterian animals, but can be found among the cnidarians. One interpretation is that the common ancestor of cnidarians and bilaterians had a muscular worm-shaped body plan. However, this does not seem compatible with the ultrastructure of *Buddenbrockia* or the phylogenetic distribution of vermiform animals. Instead, we hypothesize that the muscular, motile worm form evolved independently within cnidarians, by means of a loss of the opening to the gastrovascular cavity and subsequent acquisition of a hydrostatic skeleton. Parallel evolution of the vermiform body may have exploited a conserved developmental system for patterning an ancestral mesodermal layer homologous between Bilateria and Cnidaria. (*27*)

Ultrastructural studies reveal that the four blocks of well-defined longitudinal muscles in *Buddenbrockia* are radially distributed (Fig. 1) (*12*). Hence, *Buddenbrockia* is a tetraradial worm with two axes of symmetry across a transverse section, not a bilaterally symmetrical worm with one axis of symmetry. Bilateral symmetry was long thought to be associated with the evolution of directed locomotion, perhaps in an ancestral bilaterian. This view is challenged by the existence of subtle bilateral symmetry in sessile anthozoan cnidarians (*28*, *29*); hence, it has been suggested that bilateral symmetry arose through selection for effective internal circulation not directed locomotion (*30*). The finding that an active muscular worm evolved within the Cnidaria, yet retained radial symmetry, is consistent with this view, because it further dissociates locomotion from symmetry. *Buddenbrockia* is a worm, but not as we know it.

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Materials and Methods
Figs. S1 to S4
Tables S1 to S3
References

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Genetic Properties Influencing the Evolvability of Gene Expression

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Identifying the properties of gene networks that influence their evolution is a fundamental research goal. However, modes of evolution cannot be inferred solely from the distribution of natural variation, because selection interacts with demography and mutation rates to shape polymorphism and divergence. We estimated the effects of naturally occurring mutations on gene expression while minimizing the effect of natural selection. We demonstrate that sensitivity of gene expression to mutations increases with both increasing trans-mutational target size and the presence of a TATA box. Genes with greater sensitivity to mutations are also more sensitive to systematic environmental perturbations and stochastic noise. These results provide a mechanistic basis for gene expression evolvability that can serve as a foundation for realistic models of regulatory evolution.

Regulatory variation underlies much of phenotypic diversity, and gene expression is the first step in making ecologically and evolutionarily relevant phenotypes. Differences among genes both in standing genetic variation and in interspecies divergence in gene expression have been linked to their particular roles in biological networks (*1*–*4*) and may reflect a history of selection. However, the influence of specific evolutionary forces cannot be inferred solely from the distribution of natural variation, because selection interacts with demography and mutation to shape polymorphism and divergence (*5*). Measuring the effects of spontaneous mutations without the confounding effect of natural selection makes it possible to isolate the contribution of mutation to natural variation and is a fundamental step toward building models for the evolution of gene expression. The relationship between divergence and muta-

tional effects on gene expression has been measured in the fruit fly *Drosophila melanogaster* and the worm *Caenorhabditis elegans* (*6*, *7*), revealing that stabilizing selection plays a dominant role in limiting the extent of polymorphisms in gene expression in nature (*8*). We used *Saccharomyces cerevisiae* to investigate how the structural properties of genes and regulatory networks shape the relation between mutations and gene expression and thereby affect the course of evolution.

We performed a mutation-accumulation (MA) experiment (Fig. 1A) in *S. cerevisiae* in order to isolate the contribution of the mutational process to gene expression evolution. With serial transfer of random colonies, we accumulated spontaneous mutations by maintaining parallel lines with effective population sizes of ~10 individuals. The lines diverged from an isogenic common ancestor for 4000 generations. At this population size, the fate of most nonlethal mutations is largely governed by random genetic drift (*9*), and the divergence observed among the lines allows us to estimate the rate at which gene expression would evolve in the near absence of selection. Lethal mutations would be eliminated through our experimental protocol, but they are unlikely to contribute to standing genetic variation produced by mutations in natural populations. We randomly selected four MA lines, measured their gene expression levels with DNA microarrays (*10*), and estimated rates of gene expression evolution.

The rate of phenotypic evolution due to mutation alone can be measured by the mutational variance (V_m), which is defined as the increase in the variance of a trait introduced by mutations each generation. It can be calculated from the variance of traits among MA lines. For haploid asexual organisms, $V_m = 2\sigma^2_b/t$, where σ^2_b is the between-line variance and t is the number of generations (*5*). We estimated the V_m of gene expression for genes that showed significant statistical differences (Bayesian posterior probability > 0.99) in expression among any pair of the four MA lines by using log-transformed relative expression levels (Fig. 1B). This resulted in 2031 genes differentially expressed across strains, with 85 showing differences higher than threefold (Fig. 1C). We found that the median V_m in gene expression in yeast is 4.7×10^{-5} (fig. S1), which is comparable to that previously estimated in fruit flies [$\sim 2 \times 10^{-5}$ (*6*)] and about two orders of magnitude below those typically observed for morphological phenotypes (*11*). Hence, there are common characteristics that determine the mutational variation in gene expression in spite of large differences between these organisms. Furthermore, our estimates of V_m correlate positively with genetic variation in gene expression among natural isolates of *S. cerevisiae* (*12*) ($\rho = 0.25$, $P < 2.2 \times 10^{-16}$, $n = 1888$). Therefore, variation in levels of expression among genes and regulatory pathways in natural populations are shaped in part by variation in the transcriptional sensitivity to mutations. Also, we found that genes with high V_m tend to be underrepresented in biological processes such as cell growth and maintenance, metabolic process, cell cycle, and transcription (fig. S2).

Three main factors influence the probability that a mutation affects the expression level of a gene: (i) the number of other genes that influence the expression of the focal gene (trans-mutational

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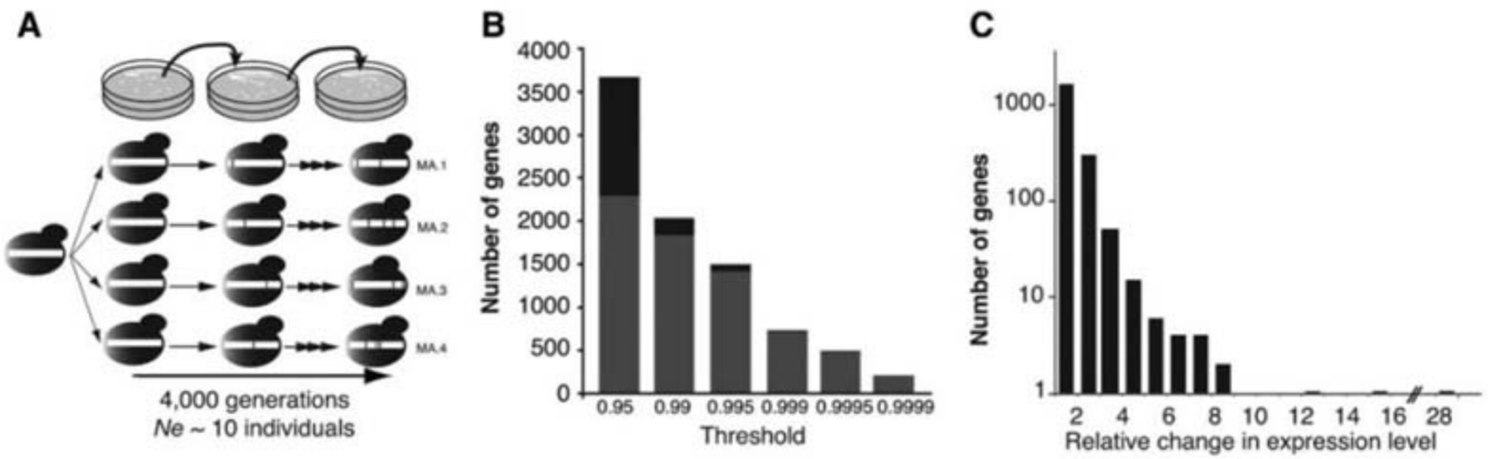

Fig. 1. (**A**) MA experimental design. (**B**) Number of genes differentially expressed among the four MA lines as a function of the Bayesian posterior probability of differential expression. Black bars indicate the estimated fraction of genes expected by chance. (**C**) Relative-fold change in expression level for genes with significant differences among the four MA lines.

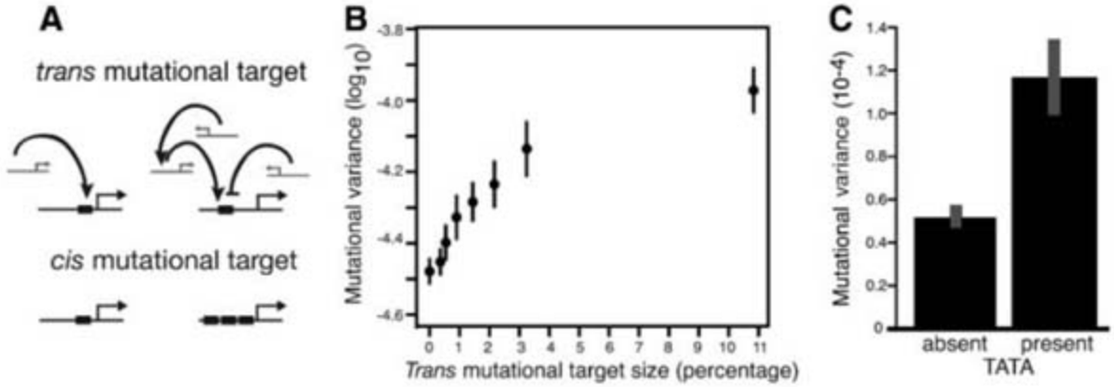

Fig. 2. (**A**) Schematic of trans- and cis-mutational target sizes. On the left of each image are cases of smaller mutation target sizes, and on the right are larger mutational target sizes. The trans-mutational target size does not solely include transcription factors but all genes acting upstream of the focal gene. (**B**) Positive relationship between trans-mutational target size and V_m. The averages of 10 bins are plotted, with error bars denoting two standard errors. (**C**) Mean V_m of genes with and without a TATA box in their promoters. Error bars denote two standard errors.

target size), (ii) the number of regulatory elements controlling the expression of the gene (cis-mutational target size), and (iii) the distribution of effects of mutations on expression. We examined whether features of these first two components could affect the sensitivity of expression levels to mutation (Fig. 2A).

The trans-mutational target size of a gene is composed of the number of genes in the genome that affect the expression level of the focal gene, weighted by their influence and their own mutational parameters (Fig. 2A). We used expression profiling of 297 gene knockouts (*13*, *14*) to estimate the trans-mutational target size of a gene as the fraction of deletions of other genes in the genome that affect its expression level. We found that V_m correlates strongly with the trans-mutational target size ($\rho = 0.33$, $P < 2 \times 10^{-16}$, $n = 1951$) (Fig. 2B and fig. S3). Hence, larger trans-mutational target sizes may indeed result in higher sensitivities of gene expression to mutations.

The cis-mutational target size of a gene scales with the number and sizes of transcription factor binding sites, either directly through the number of nucleotides in the sites or indirectly through the number and variety of regulatory molecules binding to these sites. We mapped transcription factor binding sites to yeast promoters and determined the number of binding sites per promoter (*10*, *15*). Genes that changed significantly in expression among the MA lines had a larger number of binding sites than those that did not change significantly (2.9 versus 2.4, Wilcoxon rank test, $P = 1 \times 10^{-5}$), and the V_m globally increased with the number of binding sites ($\rho = 0.14$, $P = 0.0007$, $n = 608$). Genes with a large number of transcription factor binding sites are more sensitive to spontaneous mutations affecting the level of gene expression.

Eukaryotic genes differ in the composition of their cis-regulatory targets. About one-fifth of yeast genes contain a TATA box (*16*), which modifies several aspects of their transcriptional regulation (*17*, *18*). TATA-containing genes are more likely to be subtelomeric, highly regulated by nucleosomes and chromatin regulators (*16*), and associated with elevated rates of gene-expression divergence among species (*4*) and adaptation during experimental evolution (*16*). This divergence may be the result of diversifying selection (*4*, *16*), but it could also reflect a bias in the sensitivity of TATA-containing genes to spontaneous mutations.

We found that genes with a TATA box were significantly more likely to change in expression among the MA lines (49% versus 32%; Fisher's exact test, $P = 2.5 \times 10^{-16}$) (table S1) and had a mean V_m that was twice as high as that of genes lacking a TATA box (V_m of 1.17×10^{-4} versus 0.52×10^{-4}; Wilcoxon rank test, $P < 2 \times 10^{-16}$) (Fig. 2C). Although stress-response genes are particularly enriched for TATA boxes (*4*, *18*), eliminating stress-response genes from the analysis did not change the result (V_m of 1.0×10^{-4} versus 0.51×10^{-4}; Wilcoxon rank test, $P < 2.2 \times 10^{-16}$). Hence, genes with a TATA box are more sensitive to genetic perturbations, and their over-representation among genes responding rapidly to artificial selection (*16*) and genes that show increased divergence among species (*4*) can be partly explained by their higher regulatory evolvability. Indeed, the larger trans-mutational target sizes of TATA-containing genes (0.02 versus 0.007; Wilcoxon rank test, $P = 3 \times 10^{-16}$) suggest a mechanism by which this may be achieved.

Because TATA box–containing genes have large cis- and trans-mutational target sizes relative to TATA-less genes, we used a series of generalized linear models to simultaneously assess the effects of the trans- and cis-mutational target sizes and the presence of a TATA box on the sensitivity of expression levels to mutations. First, we found that the larger number of binding sites in the promoters of TATA-containing genes [(*4*); in our data set, 3.3 versus 2.2; Wilcoxon rank test, $P = 7 \times 10^{-11}$] could fully account for the previous correlation between cis-mutational target size and V_m. When other factors are considered simultaneously, the number of transcription factor binding sites has no effect on the V_m of the gene (0.5% of the variance explained, $P = 0.11$). Second, we found that the larger trans-mutational target size of TATA-containing genes cannot fully account for the relationship between trans-mutational target size and V_m. Instead, trans-mutational target size and V_m are associated even when the effects of the TATA box are first removed (tables S2 and S3). Furthermore, we find a significant correlation between the V_m and the trans-mutational target size even after excluding TATA-containing genes ($\rho = 0.2$, $P < 0.00001$, $n = 811$), thus lending unambiguous support to the conclusion that effects of trans-mutational target size are independent of the TATA box.

A fundamental feature of organisms is their capability to cope with genetic and environmental perturbations (*19*). Whereas genetic and environmental canalization have often been conceptualized (*20*) and modeled (*21*) as distinct phenomena, mechanisms that produce canalization may act simultaneously to modulate the effects of both kinds of perturbations (*22*). Hence, phenotypes that are buffered against the effects of environmental perturbations might also be buffered against the effects of mutations. With public data on the amount of variation in gene expression over different environmental conditions (*4*), we found that V_m in the expression of a gene and its transcriptional plasticity to macro-environmental perturbations are positively correlated (Fig. 3A) ($\rho = 0.37$, $P = 2 \times 10^{-16}$, $n = 1735$). Furthermore, we found that protein expression noise, a measure of the sensitivity of gene expression to microenvironmental variation such as fluctuations in the amount of upstream cellular components (*23*–*25*), and V_m are also positively correlated (Fig. 3B) ($\rho = 0.27$, $P = 1 \times 10^{-14}$, $n = 776$). These relationships are not confounded by the effects of gene expression level, because neither mRNA nor protein abundances correlate with V_m (fig. S4, A and B). Hence, the effects of mutational and both environmental perturbations and stochastic noise are related such that mechanisms that evolve to promote or buffer transcriptional responses to one source of variation may also affect the others. Lastly, the strength of the relationship between environmental and genetic perturbations vary across sets of genes (table S4 and SOM), indicating that the relative contributions of these sources of perturbation toward the evolution of canalization may differ substantially from one gene or metabolic network to the next.

We show that not all genes are equally sensitive to the effects of random spontaneous mutations and identify structural properties (presence of a TATA box and trans-mutational target sizes) that greatly influence a gene's potential to undergo regulatory change. These determinants provide a mechanistic basis to serve as a foundation for more-realistic models of gene expression evolution that account for levels of polymorphism and divergence in cis and trans gene regulation.

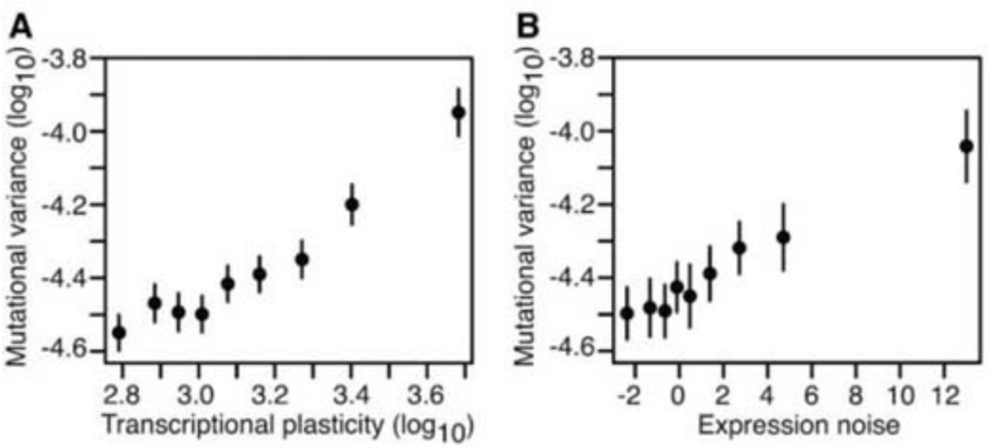

Fig. 3. Mutational variance of gene expression correlates with plasticity of transcriptional response (**A**) and stochastic noise in protein abundance (**B**). In each case, the averages of 10 bins of equal sizes are plotted, with error bars denoting two standard errors.

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Supporting Online Material

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Materials and Methods
SOM Text
Figs. S1 to S6
Tables S1 to S4

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Gender Disparity in Liver Cancer Due to Sex Differences in MyD88-Dependent IL-6 Production

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Hepatocellular carcinoma (HCC), the most common liver cancer, occurs mainly in men. Similar gender disparity is seen in mice given a chemical carcinogen, diethylnitrosamine (DEN). DEN administration caused greater increases in serum interleukin-6 (IL-6) concentration in males than it did in females. Furthermore, ablation of IL-6 abolished the gender differences in hepatocarcinogenesis in mice. DEN exposure promoted production of IL-6 in Kupffer cells (KCs) in a manner dependent on the Toll-like receptor adaptor protein MyD88, ablation of which also protected male mice from DEN-induced hepatocarcinogenesis. Estrogen inhibited secretion of IL-6 from KCs exposed to necrotic hepatocytes and reduced circulating concentrations of IL-6 in DEN-treated male mice. We propose that estrogen-mediated inhibition of IL-6 production by KCs reduces liver cancer risk in females, and these findings may be used to prevent HCC in males.

Hepatocellular carcinoma (HCC), the most common primary liver cancer, is a dreaded complication of chronic liver disease that occurs in the setting of risk factors such as hepatitis B (HBV) and hepatitis C (HCV) viral infections, alcoholic liver disease, hemochromatosis, and nonalcoholic steatohepatitis (*1*). Most HCC appears in cirrhotic livers after years of chronic inflammation. The 5-year survival rate for patients with HCC, the increasing incidence of which is likely due to the spread of HCV (*2*), is only about 7%. Notably, men are about three to five times more likely to develop HCC than women (*3*). A similar or even more pronounced gender disparity is seen in rodent HCC models (*4*, *5*). Furthermore, administration of estrogens to male mice inhibits development of chemically (DEN)–induced HCC (*6*). Nonetheless, the mechanisms that account for this gender disparity and the anticarcinogenic activity of estrogens are unknown.

Inflammation is a major contributing factor to carcinogenesis (*7*). HCC represents a classic case of inflammation-linked cancer (*8*), and chemically or genetically induced HCC depends on inflammatory signaling (*5*, *9*, *10*). To understand the mechanisms underlying gender disparity in HCC, we used the chemical carcinogen diethylnitrosamine (DEN), which causes HCC in 100% of male mice but only in 10 to 30% of female littermates (*5*, *6*). The pathogenesis of HCC in this mouse model differs from that in humans and thus may not be directly comparable to human HCC. Nevertheless, the mouse model of DEN-induced HCC has a histology and genetic signature similar to that of human HCCs with poor prognosis (*11*) and recapitulates a dependence on inflammation and gender disparity seen in human HCC.

Interleukin-6 (IL-6) is a multifunctional cytokine largely responsible for the hepatic re-

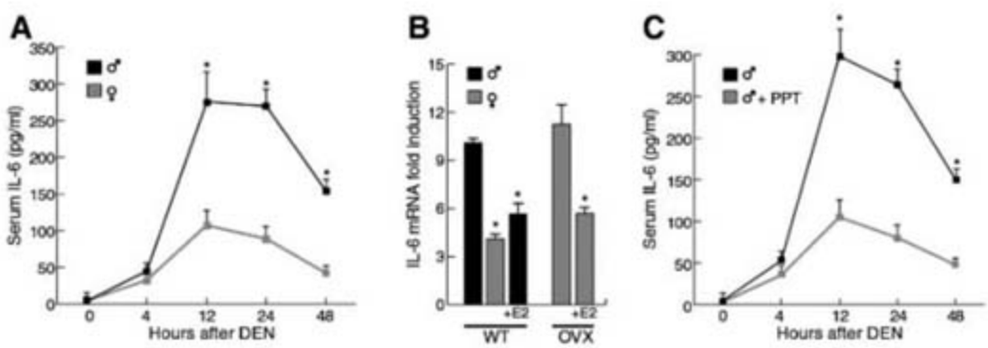

Fig. 1. Differential IL-6 production after chemically induced liver injury. (**A**) Concentration of IL-6 in serum of male and female WT mice after injection of DEN (100 mg per kg of body weight; $n = 3$ mice per time point). (**B**) IL-6 mRNA levels in livers of male, female, or ovariectomized (OVX; ovariectomy was done 2 weeks before DEN administration) female mice 4 hours after DEN injection. E2 (50 μg/kg) in corn oil was injected intraperitoneally 2 hours before DEN was administered. (**C**) Male B6 mice ($n = 3$) were injected with ERα-specific agonist propyl-pyrazole-trisphenol (PPT; 5 μg/kg in corn oil) 2 hours before DEN injection, and serum IL-6 was measured at the indicated times after DEN injection. Results in (A) to (C) are means ± SE. Asterisks indicate a significant ($P < 0.05$; Student's t test) difference relative to WT male mice.

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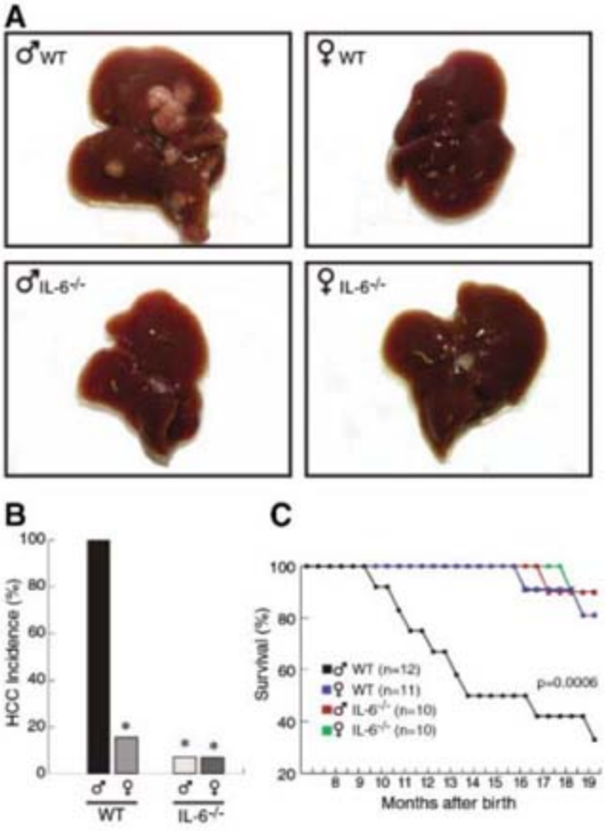

Fig. 2. Lower incidence of HCC tumors and longer survival of IL-6$^{-/-}$ mice. (**A**) Livers of 8-month-old DEN-treated mice. Multiple HCCs are seen only in WT male liver. (**B**) Incidence of HCC (>0.5 mm) in WT male (n = 14), WT female (n = 13), IL-6$^{-/-}$ male (n = 14), and IL-6$^{-/-}$ female (n = 15) mice 8 months after DEN (25 mg/kg) injection. Asterisks indicate significant (P < 0.05; Student's t test) differences relative to WT male mice. (**C**) Survival curves of WT and IL-6$^{-/-}$ mice injected with DEN (25 mg/kg) at 15 days of age (P = 0.0006; log-rank test for significance).

sponse to infections or systemic inflammation, often termed the "acute phase response." Concentrations of IL-6 in serum are increased in situations of chronic liver inflammation including alcoholic hepatitis, HBV and HCV infections, and steatohepatitis, conditions that may lead to development of HCC (*12*). IL-6 concentrations are also increased in patients with HCC relative to normal subjects (*13*). Whether IL-6 is causal or contributory to HCC is unknown. However, IL-6 is thought to contribute to hepatocyte proliferation (*14*), and DEN administration to male mice results in IL-6 production that depends on IκB kinase β (IKKβ) in myeloid cells, most likely the resident liver macrophages called Kupffer cells (KCs). In addition to preventing IL-6 production, ablation of IKKβ in myeloid cells prevents compensatory hepatocyte proliferation (*5*), a response triggered by hepatocyte death.

Compensatory proliferation appears to have a critical role in DEN-induced hepatocarcinogenesis (*5*, *10*), and IL-6 is necessary for normal liver regeneration (*14*), so we examined gender effects on DEN-induced IL-6 production (*15*). Administration of DEN resulted in higher amounts of circulating IL-6 in males than in females (Fig. 1A). A similar gender bias was seen for accumulation of IL-6 mRNA in liver (Fig. 1B). Ad-

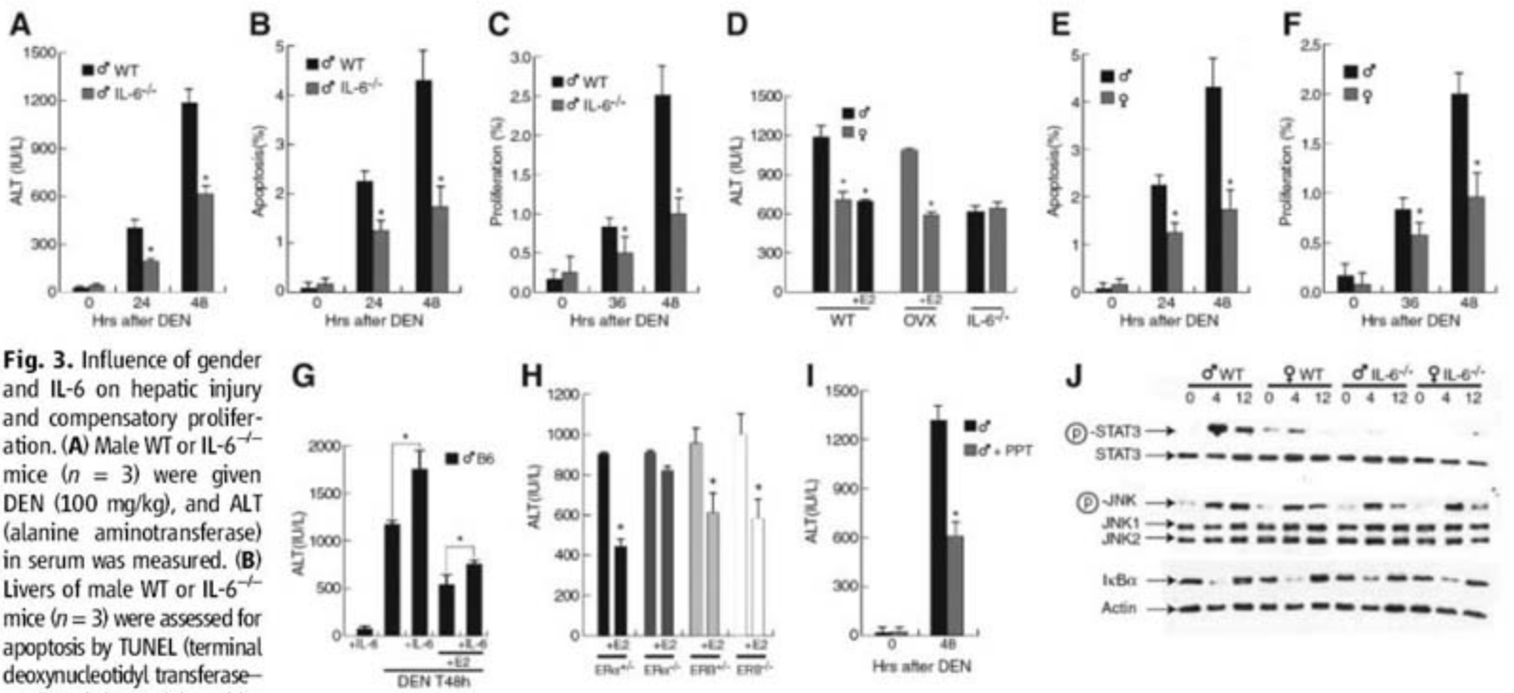

Fig. 3. Influence of gender and IL-6 on hepatic injury and compensatory proliferation. (**A**) Male WT or IL-6$^{-/-}$ mice (n = 3) were given DEN (100 mg/kg), and ALT (alanine aminotransferase) in serum was measured. (**B**) Livers of male WT or IL-6$^{-/-}$ mice (n = 3) were assessed for apoptosis by TUNEL (terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling) staining after DEN injection. (**C**) Hepatocyte proliferation in livers of DEN-injected male WT or IL-6$^{-/-}$ mice (n = 3) was assessed by injecting mice with bromodeoxyrudine (BrdU) (1 mg per mouse) 2 hours before the liver was removed. BrdU-positive cells were identified by immunostaining. (**D**) Serum ALT was measured 48 hours after DEN injection (n = 3 per group). OVX: female mice ovariectomized 2 weeks before DEN administration. E2 (50 μg/kg) in corn oil was injected 2 hours before DEN. Similar studies assessing differences between male and female mice (n = 3) were done for apoptosis (**E**) and proliferation (**F**). (**G**) Six-week-old male B6 mice (n = 3) were given E2 or vehicle (corn oil) 2 hours before DEN injection. Recombinant IL-6 (10 μg) or sham buffer (phosphate-buffered saline) was given subcutaneously at the time of DEN administration. Serum ALT was measured 48 hours later. (**H**) Male ERα$^{-/-}$ and ERβ$^{-/-}$ mice and littermate heterozygote controls (n = 3) were injected with E2 (50 μg) in corn oil or vehicle 2 hours before DEN injection, and serum ALT was measured 50 hours later. (**I**) Male mice (n = 3 per point) were injected with PPT (5 mg/kg in corn oil) or vehicle 2 hours before DEN injection, and serum ALT was measured 50 hours later. All results for (A) to (I) are means ± SE, and asterisks indicate P < 0.05 (Student's t test). (**J**) Cells from livers of male, female, and IL-6$^{-/-}$ mice were lysed at the indicated times after DEN injection. STAT3 and JNK activation, and IκBα degradation, were assessed by separating with SDS–polyacrylamide gel electrophoresis and immunoblotting with antibodies to the indicated proteins. Phosphorylation (P) of STAT3 and JNK indicates activation. Phospho-STAT3 and STAT3 were from one gel, as were Phospho-JNK and JNK1/2, and IκBα and actin.

ministration of estradiol (E2) to male mice reduced IL-6 mRNA abundance, whereas ovariectomy augmented accumulation of IL-6 mRNA in females. The latter was largely prevented by E2 administration (Fig. 1B), as well as by the estrogen receptor α (ERα) agonist propylpyrazole-trisphenol (PPT) (Fig. 1C). No gender differences were seen in IL-6 expression or hepatocyte proliferation after partial hepatectomy (fig. S1, A and B).

Pronounced gender-specific differences in IL-6 production were also seen in mice treated with carbon tetrachloride (CCl_4), a promoter of HCC development (fig. S2) (*16*). The cytotoxic effects of DEN and CCl_4 are dependent on their metabolic activation within the hepatocyte by cytochrome P450 2E1 (CYP 2E1) (*17*). Expression of CYP 2E1 did not differ between males and female mice treated with DEN (fig. S3A). Once activated, DEN forms DNA adducts (*18*). DEN-induced DNA modification and damage should lead to activation of the p53-mediated genomic surveillance response. Indeed, DEN administration led to rapid increase in expression of the p53 target genes p21 and Mdm2, but the response was practically identical in males and females (fig. S3B).

To determine whether the gender bias in IL-6 production accounts for the sex difference in HCC development, we examined DEN-induced hepatocarcinogenesis in male and female IL-6 knockout (IL-$6^{-/-}$) mice and wild-type (WT) controls. All male WT mice developed HCC, as did 13% of WT females (Fig. 2, A and B). A marked reduction in HCC incidence was seen in IL-$6^{-/-}$ males, whereas no difference was seen between WT and IL-$6^{-/-}$ females. In a cohort of mice monitored for survival, WT male mice exhibited shorter mean survival times than IL-$6^{-/-}$ males or females of either genotype (Fig. 2C).

We examined the role of IL-6 in gender differences in short-term responses elicited by DEN. Compared to WT animals, IL-$6^{-/-}$ males displayed significantly less hepatic injury after DEN administration as evidenced by reduced alanine aminotransferase (ALT) release (Fig. 3A), less apoptosis (Fig. 3B), and less necrosis (fig. S4A). Differences in compensatory proliferation matched the degree of injury, such that IL-$6^{-/-}$ males exhibited fewer proliferating hepatocytes than WT counterparts at 36 and 48 hours after DEN administration (Fig. 3C). Treatment of male mice with an antagonistic antibody that blocks IL-6 receptor signaling also provided protection from DEN-induced liver injury (fig. S5).

Consistent with previous publications (*19*, *20*), DEN-induced liver injury was reduced in females or males given E2 2 hours before DEN administration (Fig. 3D). Injury was increased in ovariectomized females and reduced after E2 administration. Absence of IL-6 eliminated gender-related differences by reducing the extent of injury in males (Fig. 3D). DEN-induced apoptosis (Fig. 3E), necrosis (fig. S4B), and compensatory proliferation (Fig. 3F) were greater in male than in female mice. Administration of exogenous IL-6 augmented DEN-induced damage in both untreated and E2-treated male mice (Fig. 3G). The reduction of injury by E2 in IL-6–treated mice suggests that E2 may also attenuate downstream IL-6 signaling. Similar gender-related differences in liver injury were seen after administration of CCl_4 (fig. S2).

Using mice deficient in either ERα or ERβ, we found that ERα is the receptor responsible for the protective effect of E2 (Fig. 3H), which was confirmed by decreased liver injury in male mice pretreated with the ERα-specific agonist PPT (Fig. 3I).

Another estrogen analog, tamoxifen, was found to act as a weak antagonist in this system and increase liver injury (fig. S6A). Absence of ERα also increased DEN-induced injury in females (fig. S6A).

IL-6 activates the transcription factor STAT3 (*21*). The activated form of STAT3 was absent in livers of IL-$6^{-/-}$ mice, and WT female mice exhibited less STAT3 activation than males after DEN administration (Fig. 3J). Female mice or IL-$6^{-/-}$ mice of both genders also exhibited reduced activation of the mitogen-activated protein kinase JNK (c-Jun N-terminal kinase) at 12 hours after DEN administration, whereas little if any difference was seen in DEN-induced IκBα degradation. Sustained activation of JNK is required for DEN-induced liver injury as well as hepatocarcinogenesis (*5*, *10*).

Estrogens inhibit IL-6 promoter activity by decreasing the activity of the transcription factors nuclear factor κB (NF-κB) and C/EBPβ (*22*). KCs from male mice produced IL-6 when incubated with either bacterial lipopolysaccharide (LPS) or cellular debris released by necrotic hepatocytes (Fig. 4A). Both responses were strongly dependent on IKKβ or the Toll-like receptor (TLR) adaptor protein MyD88 and were inhibited if the KCs were first incubated with E2 (Fig. 4A). We speculated that necrotic debris released by DEN-injured hepatocytes triggers cytokine production and compensatory proliferation (*5*). The TLR adaptor MyD88, which was required for IL-6 induction by necrotic debris, was also required for DEN-induced production of IL-6 in vivo (Fig. 4B) and for induction of liver injury (Fig. 4C). MyD88 was also required for optimal CCl_4-induced accumulation of IL-6 mRNA (fig. S7). MyD88 is also required for induction of liver injury in response to hypoxia (*23*) and LPS (*24*). Furthermore, MyD88 ablation suppressed DEN-induced hepatocarcinogenesis. MyD88$^{-/-}$ male mice developed fewer (Fig. 4D) and smaller (Fig. 4E) HCC tumors than WT male mice.

Administration of DEN also leads to modest accumulation of tumor necrosis factor–α (TNF-α) mRNA (*5*), another proinflammatory cytokine thought to be involved in liver regeneration (*25*). However, TNF-α expression did not exhibit gender-dependent differences (fig. S8A), and ablation of TNF-α or its type 1 receptor (TNFR1) had little if any effect on production of IL-6 in response to DEN (fig. S8B). Thus, IL-6 induction and liver injury are dependent on signaling via MyD88 but not through TNFR1. Accordingly, ablation of TNFR1 had no significant effect on DEN-induced hepatocarcinogenesis (fig. S8, C and D).

Our results explain why females are less prone to liver cancer than males. This study and others (*5*, *10*) show a strong correlation between the amount of liver damage during acute toxicity and inflammation and the extent of HCC development. We found that both liver injury and

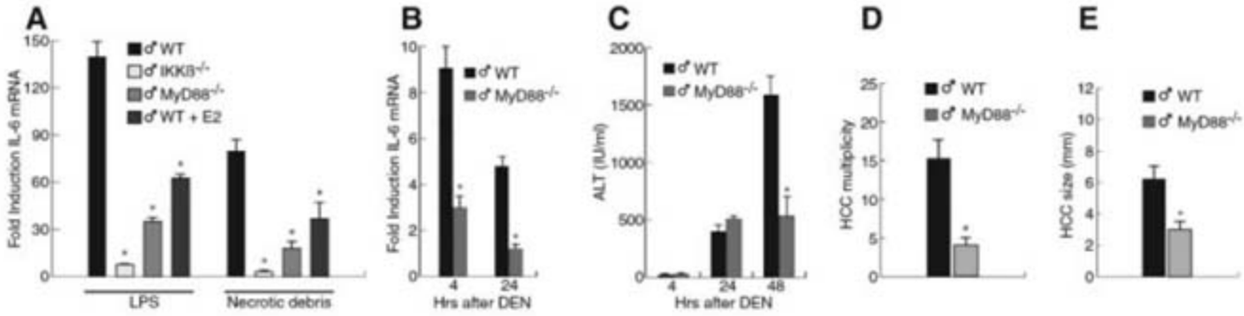

Fig. 4. Requirement of MyD88 for IL-6 production, injury, and hepatocarcinogenesis after DEN treatment. (**A**) Accumulation of IL-6 mRNA was measured by real-time polymerase chain reaction in KCs from male WT, IKKβ$^{-/-}$, or MyD88$^{-/-}$ mice ($n = 3$ experiments per time point) after incubation with LPS (10 ng/ml) or necrotic debris prepared by cycles of freeze-thawing of primary hepatocytes. Where indicated, cells were incubated with E2 (10 ng/ml) 30 min before stimulation. (**B** and **C**) Male WT and MyD88$^{-/-}$ mice were injected with DEN, and liver IL-6 mRNA (B) or serum ALT (C) was measured. (**D** and **E**) Number of HCCs (D) and sizes (E) in livers of WT and MyD88$^{-/-}$ male mice 8 months after DEN (25 mg/kg) administration. Results in (A) to (E) are means ± SE. Asterisks indicate a significant ($P < 0.05$; Student's t test) difference relative to WT males.

compensatory proliferation were strongly dependent on IL-6 and that the absence of this tumor-promoting cytokine resulted in almost complete inhibition of DEN-induced hepatocarcinogenesis. IL-6 production by KCs was largely dependent on MyD88, an adaptor molecule that acts downstream of TLRs as well as IL-1 receptor (*26*). Because DEN is not a direct macrophage or KC activator (*27*), hepatocyte necrosis may be an intermediate in the pathway through which DEN or CCl_4 exposure results in IL-6 production. Various macromolecules released by necrotic cells activate macrophages through TLRs, the receptors which in turn activate MyD88 (*28*, *29*). TRIF, another TLR adaptor protein (*26*), is not required for DEN- or CCl_4-induced IL-6 production and liver injury (*27*). MyD88 signaling, but not TNFR1 signaling, was required for optimal DEN-induced hepatocarcinogenesis in male mice. DEN-induced hepatocarcinogenesis appears to depend on an inflammatory response, triggered by hepatocyte necrosis, that leads to production of IL-6. Estrogens, at concentrations present in females but not in males, suppress IL-6 production and therefore inhibit chemically induced liver carcinogenesis. A similar mechanism could account for the gender bias in liver cancer in humans. If so, estrogen-mimetic compounds capable of inhibiting excessive IL-6 production might be used to prevent progression of chronic liver disease to HCC in men.

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Supporting Online Material

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Materials and Methods
Figs. S1 to S8
References

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Regulation of Spontaneous Intestinal Tumorigenesis Through the Adaptor Protein MyD88

Seth Rakoff-Nahoum and Ruslan Medzhitov*

Inflammation is increasingly recognized as an important component of tumorigenesis, although the mechanisms and pathways involved are not well understood. Tumor development is regulated by products of several modifier genes, but instructions for their tumor-specific expression are currently unknown. We show that the signaling through the adaptor protein MyD88 has a critical role in spontaneous tumor development in mice with heterozygous mutation in the adenomatous polyposis coli (APC) gene. We found that MyD88-dependent signaling controls the expression of several key modifier genes of intestinal tumorigenesis and has a critical role in both spontaneous and carcinogen-induced tumor development. This study thus reveals the important role of an innate immune signaling pathway in intestinal tumorigenesis.

Inflammatory responses contribute to carcinogenesis through multiple mechanisms (*1*–*3*). Activation of the transcription factor nuclear factor κB (NF-κB), a key mediator of inflammation, has a critical role in the regulation of tumor development resulting from chronic inflammation or exogenous mutagens (*4*, *5*). NF-κB is activated by multiple stimuli (*6*), and it is currently unknown which pathway is critically involved in cancer-associated inflammation and the tissue repair response (*7*). The role of inflammatory and tissue repair responses in spontaneous carcinogenesis, independent of chronic inflammation or administration of exogenous carcinogens, has not yet been characterized. However, signaling through Toll-like receptors (TLRs) of the innate immune system to MyD88 (a signaling adaptor of TLRs) has a critical role in the control of tissue renewal responses (*8*–*11*).

A link between intestinal tissue renewal and tumorigenesis was established when the genetic basis of familial associated polyposis (FAP) was mapped to the APC gene (*12*). Germline and sporadic mutations in *APC* occur in >85% of FAP and >80% of sporadic colorectal tumors (*12*). A mouse model of spontaneous intestinal tumorigenesis was discovered in a forward genetic screen (*13*). These mice, designated $Apc^{Min/+}$, have a mutation in the *APC* gene and develop 60 to 80 intestinal adenomas, mostly at the distal small intestine (*14*). Given the role of the MyD88 signaling in intestinal tissue renewal and repair, we investigated the role of this pathway in spontaneous intestinal carcinogenesis in $Apc^{Min/+}$ mice.

To examine a potential role of the MyD88 signaling pathway in spontaneous intestinal tumorigenesis, we generated $Apc^{Min/+}$ mice on MyD88-sufficient and -deficient backgrounds and analyzed sex- and age-matched cohorts. On average, $Apc^{Min/+}$ mice die within 6 months of age from complications of intestinal tumors (*13*). In contrast, mortality of $Apc^{Min/+}$ $MyD88^{-/-}$ mice was dramatically reduced as compared with $Apc^{Min/+}$ littermate controls (Fig. 1A). We investigated the red blood cell (RBC) status of these mice, a marker of intestinal tumorigenesis, and found that the anemia observed in $Apc^{Min/+}$ mice was significantly ameliorated in $Apc^{Min/+}$ $MyD88^{-/-}$ mice (Fig. 1B). MyD88-dependent signaling therefore contributes substantially to the severe mortality and morbidity caused by inactivation of *APC*.

The number of visible polyps (≥0.5 mm in diameter) was next quantified by stereoscopic microscopy. The number of macroadenomas in the small intestines or colon of $Apc^{Min/+}$ $MyD88^{-/-}$ mice was reduced compared with that in $Apc^{Min/+}$ controls (Fig. 2A and fig. S1). Loss of MyD88 decreased the number of small intestinal tumors

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in all regions of the small intestine (Fig. 2B), most notably in the distal small intestine where the majority of tumor formation in $Apc^{Min/+}$ mice occurs (*13*) (Fig. 2B).

The number of polyps in $Apc^{Min/+}$ $MyD88^{-/-}$ mice was reduced, and the polyps that were present, both at the proximal and distal small intestine, were smaller in size than those present in age-matched $Apc^{Min/+}$ mice (Fig. 2B and fig. S1). This finding suggested that the MyD88-dependent signaling pathway regulated intestinal tumor growth in $Apc^{Min/+}$ mice. Because the size of tumors can be influenced by the balance of cell proliferation and death within the tumor mass, we investigated the proliferative and apoptotic rates within size-matched tumors from $Apc^{Min/+}$ and $Apc^{Min/+}$ $MyD88^{-/-}$ mice (*15*). Polyps from both genotypes showed similar proportions of proliferating cells (Fig. 3A). However, higher numbers of apoptotic cells were found in tumors from $Apc^{Min/+}$ $MyD88^{-/-}$ mice than in samples from $Apc^{Min/+}$ mice (Fig. 3B and fig. S2A). Thus, MyD88 may contribute to tumor progression through regulation of apoptosis.

Intestinal tumorigenesis is a stepwise process (*12*). At the first step, referred to as initiation, genetic changes in either oncogenes or tumor suppressors lead to the transformation of normal epithelium to dysplastic cells, resulting in the formation of microadenomas. Tumor progression results in the expansion of microadenomas and an increase in tumor size (*4*, *12*). The decrease in polyp frequency and size observed in $Apc^{Min/+}$ $MyD88^{-/-}$ compared with those in $Apc^{Min/+}$ mice indicated that MyD88 regulates initiation or the progression from microadenomas to macroadenomas. The cancer initiation step in both FAP patients and $Apc^{Min/+}$ mice involves the loss of heterozygosity of the *APC* gene and results in the formation of microadenomas (*12*). To investigate whether MyD88 affected early events in neoplasia, we analyzed $Apc^{Min/+}$ and $Apc^{Min/+}$ $MyD88^{-/-}$ mice for the presence of microadenomas. Four- to five-week-old $Apc^{Min/+}$ mice, an age at which the majority of microadenoma formation occurs (*13*), were observed to have similar frequencies [$Apc^{Min/+}$: 2.06 ± 1.63 (SEM); $Apc^{Min/+}$ $MyD88^{-/-}$: 2.19 ± 0.95; microadenomas per Swiss roll section of entire small intestine; $N = 4$ mice per genotype] and morphology of microadenomas regardless of the presence or absence of MyD88 (Fig. 3B). Thus, MyD88 appears not to influence the formation of early neoplastic lesions in intestinal tumorigenesis but rather contributes to tumor growth and progression. In addition, macroadenomas of both $Apc^{Min/+}$ and $Apc^{Min/+}$ $MyD88^{-/-}$ mice showed an equivalent increase in cytoplasmic and nuclear accumulation of β-catenin (Fig. 3D) and frequencies of cells with nuclear β-catenin (fig. S2B), suggesting that MyD88-dependent signaling does not affect β-catenin nuclear translocation in dysplastic epithelium.

We further investigated the role of MyD88 in intestinal tumorigenesis by comparing gene expression in size-matched polyps from $Apc^{Min/+}$ and $Apc^{Min/+}$ $MyD88^{-/-}$ mice. This analysis revealed two classes of genes that are overexpressed in tumors compared with normal tissue. One class of genes was MyD88-independent in that their expression was similarly upregulated in $Apc^{Min/+}$ and $Apc^{Min/+}$ $MyD88^{-/-}$ tumors (fig. S3). This class of genes included, for example, c-Myc, which is known to be a direct target of β-catenin (*16*). These tumor-upregulated, but MyD88-independent, genes serve as internal controls for tumor tissue sampling.

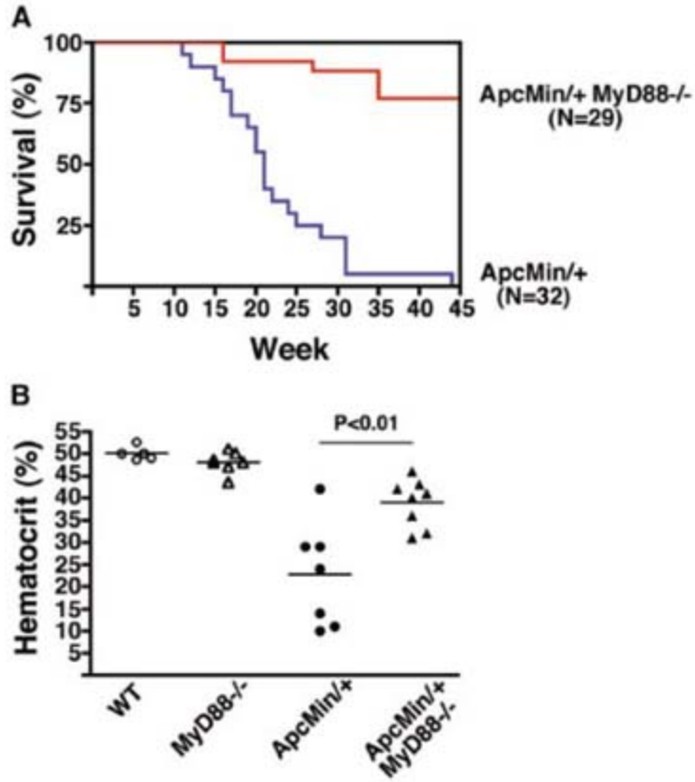

Fig. 1. Effects of MyD88-deficiency on mortality and blood loss in $Apc^{Min/+}$ mice. (**A**) $Apc^{Min/+}$ $MyD88^{+/+}$ or $MyD88^{+/-}$ ($Apc^{Min/+}$; N = 32) and $Apc^{Min/+}$ $MyD88^{-/-}$ ($Apc^{Min/+}$ $MyD88^{-/-}$; N = 29) mice were passively followed for long-term survival. Difference in survival was determined significant by the Mantel-Haenszel/Log-rank test. (**B**) At time of sacrifice, hematocrit of peripheral blood of age- and sex-matched mice (20 to 22 weeks old) was determined. Statistical analysis was performed using the Mann-Whitney U test.

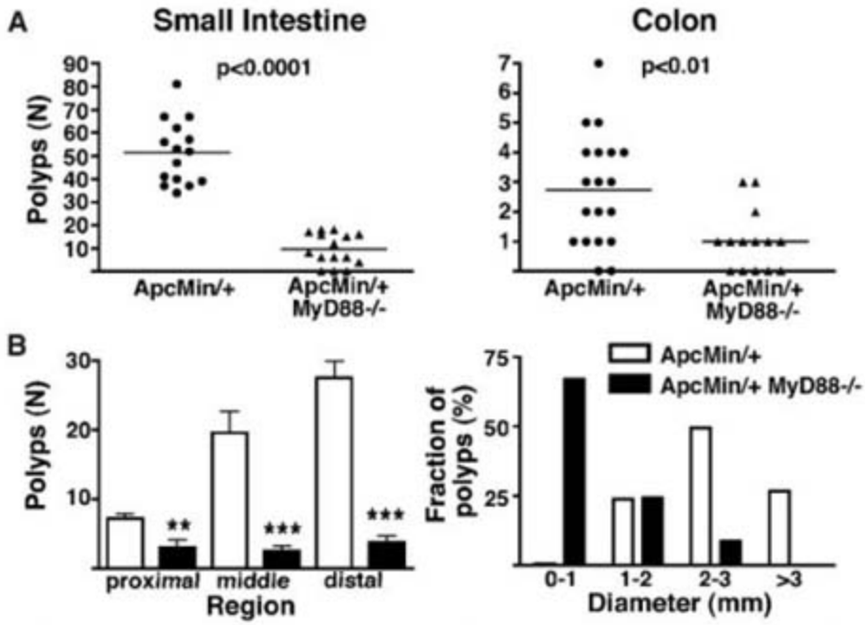

Fig. 2. Characterization of tumors in $Apc^{Min/+}$ mice in the presence or absence of MyD88. (**A**) (Left) The number of visible polyps in the small intestine (≥0.5 mm in diameter) was quantified by stereoscopic microscopy in age (20 to 33 weeks old)- and sex-matched $Apc^{Min/+}$ and $Apc^{Min/+}$ $MyD88^{-/-}$ mice. (Right) The number of visible polyps in the large intestine in age (20 to 28 weeks old)- and sex-matched $Apc^{Min/+}$ and $Apc^{Min/+}$ $MyD88^{-/-}$ mice. (**B**) (Left) Frequency of the polyps in $Apc^{Min/+}$ and $Apc^{Min/+}$ $MyD88^{-/-}$ mice stratified by small intestinal region. (Right) Size range of tumors from age- and sex-matched $Apc^{Min/+}$ and $Apc^{Min/+}$ $MyD88^{-/-}$ mice. Error bars, ±SEM. **, $P <$ 0.01; ***, $P < 0.001$ (compared with $Apc^{Min/+}$); Mann-Whitney U test.

However, in these same size-matched tumors, we found many genes that showed increased expression in a tumor-specific and MyD88-dependent manner (Fig. 4). These included genes encoding positive regulators of intestinal tumorigenesis, such as cyclooxygenase-2 (COX-2) (*17*, *18*), matrix metalloprotease (MMP) 7 (*19*), and cytosolic phospholipase A2 (cPLA2) (*20*, *21*) (Fig. 4). MyD88-dependent signaling was also required for the tumor-specific expression of genes encoding keratinocyte growth factor 2 (KGF2) [also called fibroblast growth factor 10 (FGF10)], CD44, MMP10, insulin-like growth factor binding protein (IGFBP) 5, insulin-like growth factor–1 (IGF1), regenerating gene product IIIβ (RegIIIβ), and various cytokines and chemokines such as keratinocyte derived chemokine (KC), interleukin (IL)–6, and IL-1β (Fig. 4 and fig. S4), all of which are components of the tissue repair response and have been either shown to be, or implicated as, positive regulators of tumorigenesis. Thus, MyD88-dependent signaling is critically involved in the tumor-specific induction of modifier genes of intestinal tumorigenesis, as well as genes involved in intestinal tissue repair. Interestingly, MyD88-dependent genes in $Apc^{Min/+}$ tumors included both classical NF-κB–regulated genes (KC and IL-6) and genes that are regulated by other signaling pathways (FGF10, RegIIIβ, IGF1, and IGFBP5).

The tumor modifier MMP7 is expressed only in dysplastic epithelium (*19*), whereas another tumor modifier, COX-2, is expressed by multiple cell types in $Apc^{Min/+}$ tumors, including macrophages, fibroblasts, endothelial cells, and epithelium (*22*). Overexpression of both MMP7 and

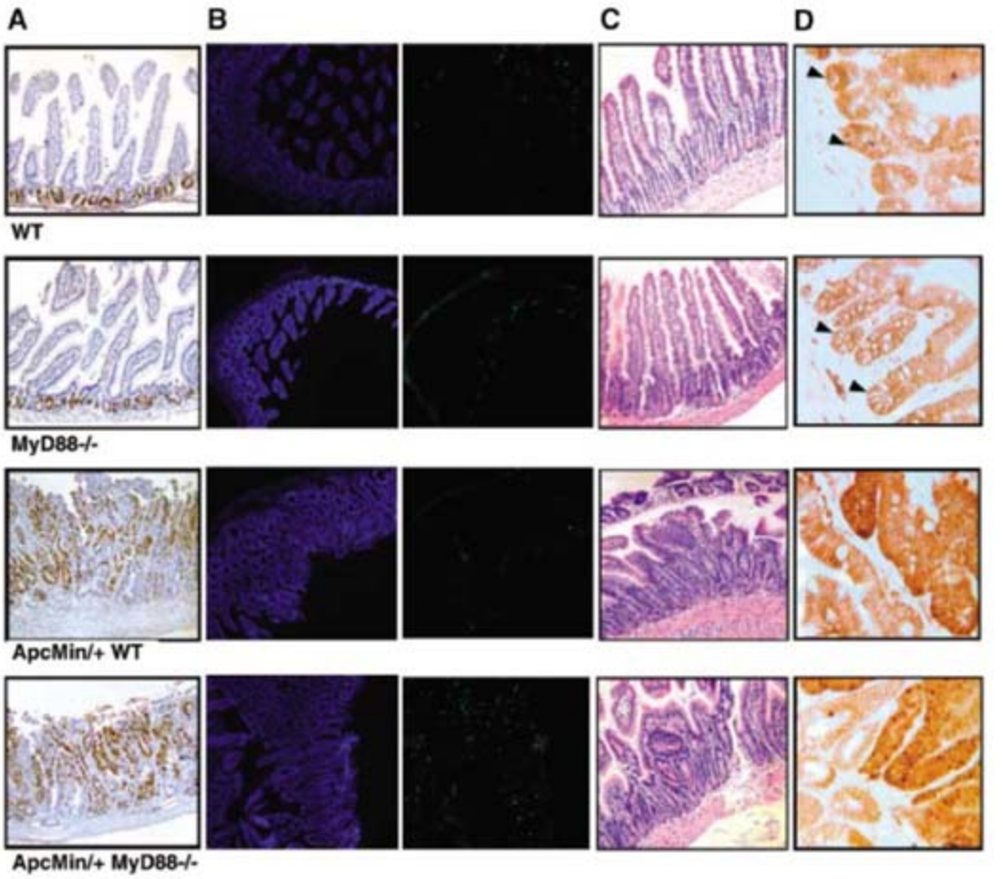

Fig. 3. MyD88-dependent and -independent epithelial homeostasis in intestinal tumors and formation of early neoplastic lesions. (**A**) 5-bromodeoxyuridine (BrDU), (**B**) (left panel) diamidino-phenylindole (DAPI), and (right panel) terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick end labeling (TUNEL) stain of intestinal tissue from WT and $MyD88^{-/-}$ mice and size-matched tumors from $Apc^{Min/+}$ and $Apc^{Min/+}$ $MyD88^{-/-}$ mice. (**C**) Representative histologic microphotos of distal small intestine from littermate mice at 4.5 weeks of age. Haemotoxylin and eosin stain; images are at 200x magnification. (**D**) Representative β-catenin staining of small intestine of age (20 weeks)- and sex-matched mice. Images are at 400x magnification. Arrowheads indicate nuclear localization of β-catenin at cells present at the crypt base in WT and $MyD88^{-/-}$ mice. Increased cytoplasmic and nuclear β-catenin staining can be seen in numerous cells in tumors of $Apc^{Min/+}$ and $Apc^{Min/+}$ $MyD88^{-/-}$ mice.

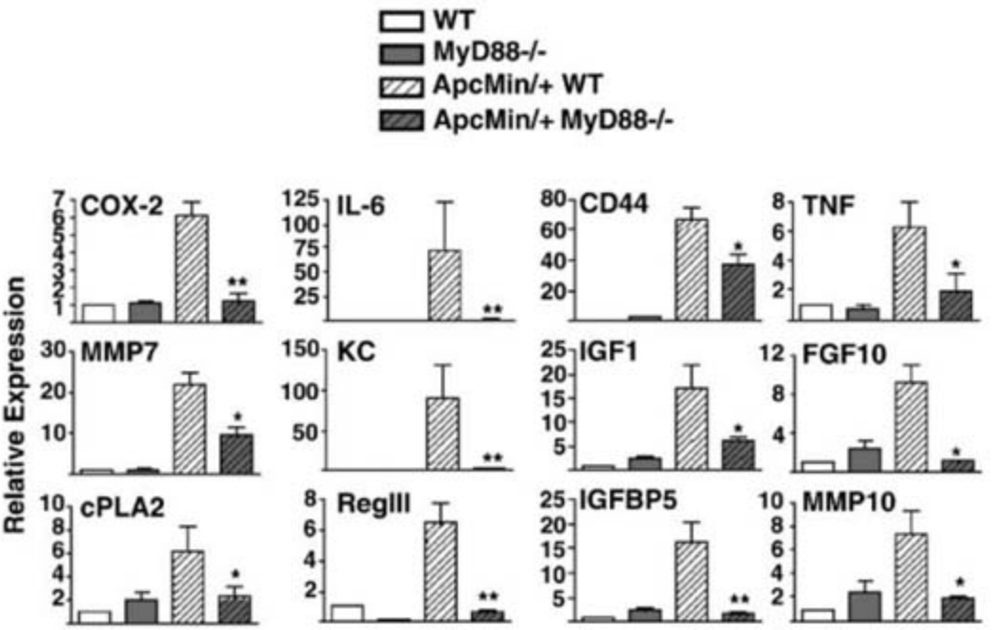

Fig. 4. MyD88 regulation of modifiers of tumor progression. RNA was prepared from the distal small intestine of normal intestine of WT and $MyD88^{-/-}$ mice and size-matched tumors of $Apc^{Min/+}$ and $Apc^{Min/+}$ $MyD88^{-/-}$ mice. cDNA was prepared, and quantitative polymerase chain reaction was performed. Expression of candidate genes was normalized to hypoxanthine-guanine phosphoribosyltransferase (HPRT) and relative induction compared with levels in the intestines of WT mice. Data are representative of five (tumor) tissue samples each isolated from different mice. Error bars, ± SEM. *, $P < 0.05$; **, $P < 0.01$ (compared to $Apc^{Min/+}$); Mann-Whitney U test.

COX-2 is MyD88-dependent in tumors (Fig. 4 and fig. S5). This suggests that MyD88 regulates intestinal tumor progression by induction of tumor modifiers in both epithelial and stromal cells, similar to the role of IKKβ-dependent signaling in chronic inflammation-dependent intestinal tumorigenesis (*4*).

We observed similar frequencies of leukocytes, such as macrophages, both in normal mucosa and different sized tumors of $Apc^{Min/+}$ mice (*23*) (fig. S6), indicating that differences in gene expression observed in $Apc^{Min/+}$ and $Apc^{Min/+}$ $MyD88^{-/-}$ tumors did not result from differences in leukocyte recruitment.

In another model of intestinal (colonic) tumorigenesis [the administration of the carcinogen azoxymethane (AOM) (*24*)], incidence of tumor formation was again decreased in $MyD88^{-/-}$ mice compared with that in wild-type (WT) mice (fig. S7). Thus, the MyD88-dependent signaling pathway critically contributes to carcinogen-induced colonic tumorigenesis as well.

MyD88 functions in signaling downstream of both TLR and IL-1 receptor families. Although both types of receptors may contribute to the phenotype of $Apc^{Min/+}$ $MyD88^{-/-}$ mice, the function of IL-1 is likely to be downstream of TLRs because expression of IL-1 is dependent on TLR-MyD88 signaling (*25*) (fig. S4). The nature of the trigger for TLR signals in $Apc^{Min/+}$ tumors is currently unknown.

The MyD88 signaling pathway triggered by the TLR/IL-1R family of receptors functions in the control of inflammation both in host defense from infection and in tissue repair (*8*, *9*, *11*, *26*). Many genes involved in tissue repair (*27*) are induced in a TLR and MyD88-dependent manner upon intestinal injury (*8*, *9*) (fig. S8). Thus, the requirement for the MyD88 signaling pathway in intestinal tumor progression could result from the essential role of this pathway in the induction of a tissue-repair program. This would support the notion that tumor growth is an abnormal form of a continuous and unregulated state of tissue repair (*12*, *28*, *29*). Indeed, unlike the self-limiting repair in normal tissues, the tumor-associated tissue-repair response both promotes and is triggered by the tumor growth, thus falling into the self-perpetuating and uncontrolled state of tissue repair.

Our findings establish that the MyD88 signaling pathway downstream of members of the TLR and IL-1R family has a critical role in intestinal tumorigenesis. Tumor-associated expression of key modifier genes, as well as many other positive regulators of intestinal tumor progression and tissue repair, are critically dependent on the MyD88 signaling pathway. It should be noted however, that there are substantial differences between human FAP and $Apc^{Min/+}$ tumorigenesis. In addition, the $Apc^{Min/+}$ model is sensitive to a variety of genetic alterations. Therefore, it will be important to determine in future studies whether the MyD88 pathway contributes to intestinal tumorigenesis in humans.

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Yeast DNA Polymerase ε Participates in Leading-Strand DNA Replication

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Multiple DNA polymerases participate in replicating the leading and lagging strands of the eukaryotic nuclear genome. Although 50 years have passed since the first DNA polymerase was discovered, the identity of the major polymerase used for leading-strand replication is uncertain. We constructed a derivative of yeast DNA polymerase ε that retains high replication activity but has strongly reduced replication fidelity, particularly for thymine-deoxythymidine 5′-monophosphate (T-dTMP) but not adenine-deoxyadenosine 5′-monophosphate (A-dAMP) mismatches. Yeast strains with this DNA polymerase ε allele have elevated rates of T to A substitution mutations. The position and rate of these substitutions depend on the orientation of the mutational reporter and its location relative to origins of DNA replication and reveal a pattern indicating that DNA polymerase ε participates in leading-strand DNA replication.

Replication of the eukaryotic nuclear genome requires DNA polymerase α to initiate synthesis at origins and to initiate synthesis of Okazaki fragments on the lagging strand, allowing DNA polymerases δ (pol δ) and ε (pol ε) to then perform the bulk of chain elongation (*1*, *2*). Pol δ is implicated in lagging-strand replication (*1*), but the identity of the polymerase(s) that replicates the leading strand is unknown (*1*, *2*). Null alleles of the *POL2* (pol ε) and *POL3* (pol δ) genes are uninformative for identifying the leading-strand polymerase, because both genes are essential for normal replication. To retain replication activity while generating a distinct mutational signature in vivo that allows assignment of pol ε to leading- and/or lagging-strand replication in yeast cells, we substituted glycine for Met^{644} at the *Saccharomyces cerevisiae* pol ε active site. Yeast pol ε with the Met^{644}Gly change retains 44% of wild-type polymerase activity (Fig. 1A) and retains full 3′ exonuclease activity (Fig. 1B). A haploid *pol2-M644G* yeast strain grows at a rate similar to a *POL2* strain (Fig. 1C), indicating that $M^{644}G$ pol ε retains substantial replicative capacity. In both its exonuclease-proficient (Fig. 1D) and exonuclease-deficient forms (Fig. 1E), $M^{644}G$ pol ε synthesizes DNA in vitro with reduced fidelity in comparison with wild-type (i.e., Met^{644}) pol ε (Fig. 1 and table S1) (*3*), i.e., it is defective in discriminating against deoxynucleotide triphosphate (dNTP) misinsertion. Even the exonuclease-

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proficient polymerase has an elevated base-substitution error rate (Fig. 1D), indicating that despite retaining proofreading potential (Fig. 1B), $M^{644}G$ pol ε does not efficiently proofread certain mismatches, for example, T-dTMP mismatches. This is more obvious in some sequence contexts than others. Among 16 positions in the *lacZ* template where T to A substitutions can be detected (fig. S1), errors are particularly prevalent at template T+147 and T–36 (Fig. 1F), resulting in a high error rate of exonuclease-proficient $M^{644}G$ pol ε for T-dTMP mismatches (11×10^{-5}). In contrast, the lowest error rate for exonuclease-proficient $M^{644}G$ pol ε is $\leq 0.28 \times 10^{-5}$ for the A-dAMP mismatch, a difference of at least 39-fold (Fig. 1D). This large difference in the rate of stable misincorporation of dTMP opposite T compared to dAMP opposite A is critical for interpreting $M^{644}G$ pol ε's distinctive mutational signature in yeast, because these mismatches are the two possible intermediates that could result in an A–T to T–A substitution in vivo.

In haploid yeast containing the exonuclease-proficient *pol2-M644G* allele, the spontaneous mutation rate at the *CAN1* locus was elevated by a factor of 3.9 compared with a wild-type strain (table S3) (*3*). When repair of single-base mismatches was inactivated by disrupting *MSH6*, the mutation rate at *CAN1* in the *pol2-M644G* strain was 58 times as high as that for the *Δmsh6* strain with wild-type *POL2* (table S3), consistent with inaccurate DNA replication in vivo by exonuclease-proficient $M^{644}G$ pol ε. When 11 independent Can^r mutants from the *pol2-M644G* strain were sequenced, six contained T–A to A–T substitutions predicted by the high rate of T-dTMP mismatch formation by $M^{644}G$ pol ε (Fig. 1D).

Based on these results, we investigated whether $M^{644}G$ pol ε participates in leading- and/or lagging-strand replication using a strategy previously employed to follow replicative mutagenesis on the two strands in strains encoding wild-type or exonuclease-deficient DNA polymerases (*4*–*6*). In those studies, the template strand for replication errors was assigned by monitoring incorporation of 6-hydroxylaminopurine monophosphate opposite template cytosine on one strand, or incorporation of dAMP opposite template 8-oxo-G on the other strand. In the present study, we assigned the template strand based on the strong preference of $M^{644}G$ pol ε for stable misincorporation of dTMP opposite T rather than dAMP opposite template A (Fig. 1D). We compared spontaneous mutation rates in strains containing the *pol2-M644G* allele or the wild-type *POL2* gene (Table 1). Rates were measured using the *URA3* reporter gene, which was inserted in each of the two possible orientations, and on opposite sides of, but close to, ARS306 (Fig. 2), an origin of replication on the left arm of chromosome III that fires in early S phase with >90% efficiency (*7*).

URA3 mutation rates in the wild-type *POL2* strains ranged from 3.1×10^{-8} to 6.6×10^{-8}, whereas rates in the *pol2-M644G* strains ranged from 16×10^{-8} to 45×10^{-8} (Table 1). When independent *ura3* mutants from these strains were sequenced, a variety of mutations were observed in the wild-type (M644) strains, consistent with many sources of mutagenesis in wild-type yeast. Among the mutations, A–T to T–A substitutions were rare, resulting in low spontaneous mutation rates for these two events in wild-type strains (Table 1). In contrast, and as

Table 1. Mutation rates at *URA3*. Mutation rates were measured as described in (*15*). Parentheses contain the numbers of A–T to T–A plus T–A to A–T substitutions observed among the total number of *ura3* mutants sequenced. CI, confidence interval.

Yeast strain	Location and origin	*URA3* orient.	Mut. rate ($\times 10^{-8}$)	95% CI	A–T to T–A plus T–A to A–T ($\times 10^{-8}$)	Occurrences A–T to T–A: T–A to A–T
Wild type	R-ARS306	1	6.6	3.5–12	1.6 (3/12)	2:1
		2	5.9	1.6–17	1.5 (2/8)	1:1
pol2-M644G	L-ARS306	1	16	11–36	5.3 (7/21)	1:6
		2	45	23–60	29 (20/31)	18:2
	R-ARS306	1	28	13–40	19 (45/61)	43:2
		2	16	9.7–36	2.1 (5/38)	1:4
Wild type	R-ARS501	1	3.9	3.2–7.2	0.16 (1/24)	0:1
		2	3.1	2.2–6.4	0.25 (2/25)	1:1
pol2-M644G	R-ARS501	1	26	17–40	17 (22/34)	22:0
		2	21	15–44	5.9 (9/32)	2:7

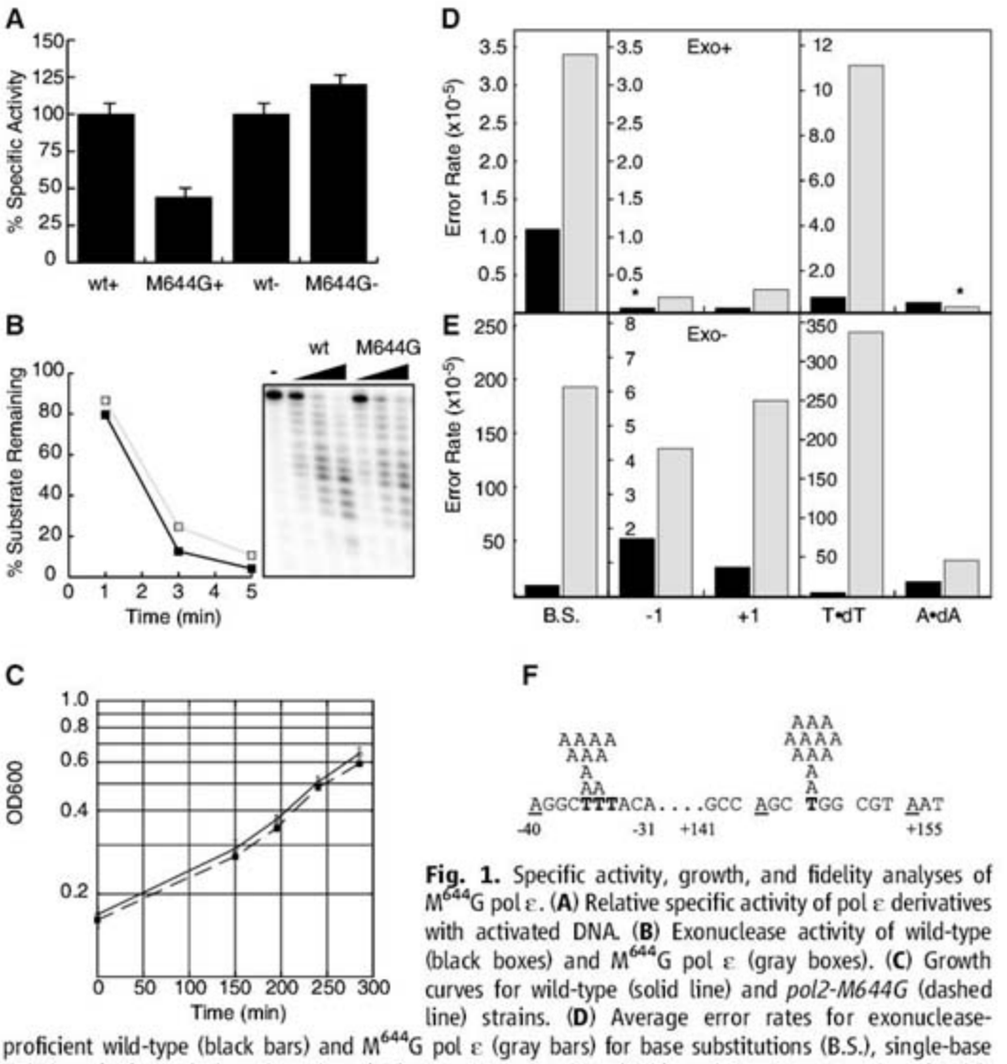

Fig. 1. Specific activity, growth, and fidelity analyses of $M^{644}G$ pol ε. (**A**) Relative specific activity of pol ε derivatives with activated DNA. (**B**) Exonuclease activity of wild-type (black boxes) and $M^{644}G$ pol ε (gray boxes). (**C**) Growth curves for wild-type (solid line) and *pol2-M644G* (dashed line) strains. (**D**) Average error rates for exonuclease-proficient wild-type (black bars) and $M^{644}G$ pol ε (gray bars) for base substitutions (B.S.), single-base deletions (–1), single-base insertions (+1), T to A transversions (*T•dT*), and A to T transversions (*A•dA*). Asterisks denote error rates that are ≤ the indicated value. (**E**) As in (D) but for exonuclease-deficient pol ε. (**F**) T to A transversions at –36 and +147 positions in *lacZ* using exonuclease-proficient $M^{644}G$ pol ε. Sites where T to A and A to T transversions are detectable are in bold and underlined, respectively.

predicted by the high rate of T-dTMP mismatch formation by $M^{644}G$ pol ε in vitro (Fig. 1D), the rates of A–T to T–A substitutions were higher in the *pol2-M644G* strains (Table 1). Just as misincorporation in vitro was prevalent at certain template locations in the *lacZ* template (e.g., T+147 and T–36, Fig. 1F), the majority of these transversions generated in the *pol2-M644G* strains occurred at two specific base pairs (hot spots) in the *URA3* coding sequence, numbers 279 and 686 (Fig. 2).

The rate of these substitutions depended strongly on the orientation and location of the *URA3* gene. When *URA3* was placed to the right of ARS306 in the *pol2-M644G* strain, 43 of 61 mutants recovered contained an A to T transversion, defined relative to the *URA3* coding strand (Table 1), and 36 of these 43 were at base pair number 686 (Fig. 2A). This yields a rate of A to T transversion at base pair 686 in orientation 1 of 13×10^{-8} (Fig. 2A). This rate is higher than that observed in the corresponding wild-type strain ($\leq 0.6 \times 10^{-8}$) (Fig. 2A), which indicates that the A to T mutations are dependent on replication by $M^{644}G$ pol ε. Because ARS306 is only ~1700 base pairs distant from the *URA3* gene, the replication fork emanating from ARS306 reaches base pair 686 long before the fork emanating from ARS307, which is over 32,000 base pairs to the right of *URA3*. In this case, if we assume from the error specificity in vitro (Fig. 1D) that these events resulted from T-dTMP mismatches generated by $M^{644}G$ pol ε rather than by A-dAMP mismatches, pol ε is inferred to replicate the leading-strand template, as depicted in Fig. 2A.

In contrast to the two A to T hot spots, only two T to A events were detected among 61 mutations recovered from this strain (Table 1, R-ARS306, orientation 1), even though there are many sites where such substitutions can be scored. Such T to A events would be inferred to result from T-dTMP mismatches generated if M644G pol ε was replicating the lagging-strand DNA template. This paucity of T to A substitutions could be due to the fact that pol ε participates much more in leading-strand replication than in lagging-strand replication. Alternatively, a hot spot sequence context may not be present in the lagging-strand template. These two possibilities were distinguished by comparing results in a second strain in which *URA3* was again placed to the right of ARS306, but now in the opposite orientation. In orientation 2, where a T-dTMP mismatch at base pair 686 would be a lagging-strand error, the rate of A to T transversions at base pair 686 was observed to be lower by a factor of at least 32 ($\leq 0.4 \times 10^{-8}$, Fig. 2B) as that in orientation 1. Thus, the lack of T to A substitutions in orientation 1 is not simply due to the absence of the appropriate hot spot sequence context in the lagging-strand template, but is most simply explained by prominent participation of pol ε in leading-strand replication as compared with lagging-strand replication.

This interpretation is reinforced by the fact that the opposite mutational asymmetry holds when *URA3* is located to the left of ARS306. Here, the rate of A to T transversions at base pair 686 was high (20×10^{-8}) in orientation 2 (Fig. 2C), where a T-dTMP mismatch by $M^{644}G$ pol ε would again be a leading-strand error, and the rate is lower by at least a factor of 25 ($\leq 0.5 \times 10^{-8}$) in orientation 1 (Fig. 2D), where a T-dTMP mismatch would be a lagging-strand error. These effects are not confined to the A–T base pair at position 686, because a similar pattern is observed at position 279 in the *URA3* gene (Fig. 2). A to T substitutions at position 279 are only seen in two of the four strains (Fig. 2, A and C), and in both cases the pattern is consistent with T-dTMP mismatches generated by $M^{644}G$ pol ε during replication of the leading-strand template. Finally, a similar pattern is observed at a second genomic location examined by inserting *URA3* in opposite orientations 850 base pairs to the right of ARS501, an origin of replication on the right arm of chromosome V that is used late in S phase (*8*). The results at ARS501 are similar to those at ARS306, in that the rate of A to T transversion at base pair 686 is 11 times as high in orientation 1 (Table 1 and Fig. 2E) compared with orientation 2 (Table 1 and Fig. 2F). Given that the nearest flanking origin is at least 10,000 base pairs to the right of ARS501, this pattern is consistent with T-dTMP mismatches generated by $M^{644}G$ pol ε during replication of the leading-strand template.

From the $M^{644}G$ pol ε bias for T-dTMP as opposed to A-dAMP errors (Fig. 1) and the patterns of mutagenesis in vivo (Fig. 2), we infer that $M^{644}G$ pol ε, and therefore likely wild-type pol ε, participates in leading-strand replication. This interpretation is consistent with evidence for pol δ participation in lagging-strand replication (*1*) and with evidence that the exonuclease activities of pol δ and pol ε edit 6-hydroxylaminopurine–induced mismatches on opposite strands (*6*). The interpretation that pol ε replicates the leading strand does not exclude its participation in lagging-strand replication under certain circumstances. The interpretation also does not exclude the possibility that pol δ might contribute to leading-strand replication. Both models for the participation of pol δ and pol ε in leading- and lagging-strand replication (*1*, *2*) may be correct, with the choice of polymerase dependent on such variables as replication timing (*9*), DNA sequence context, chromosomal organization and/or chromatin status, or various types of replicative stress. Given that pol ε is a known checkpoint sensor (*10*–*12*), our evidence that pol ε has an important role in replicating the leading strand is consistent with the idea that the status of leading-strand synthesis of replication fork progression determines whether the S phase checkpoint is activated (*13*, *14*).

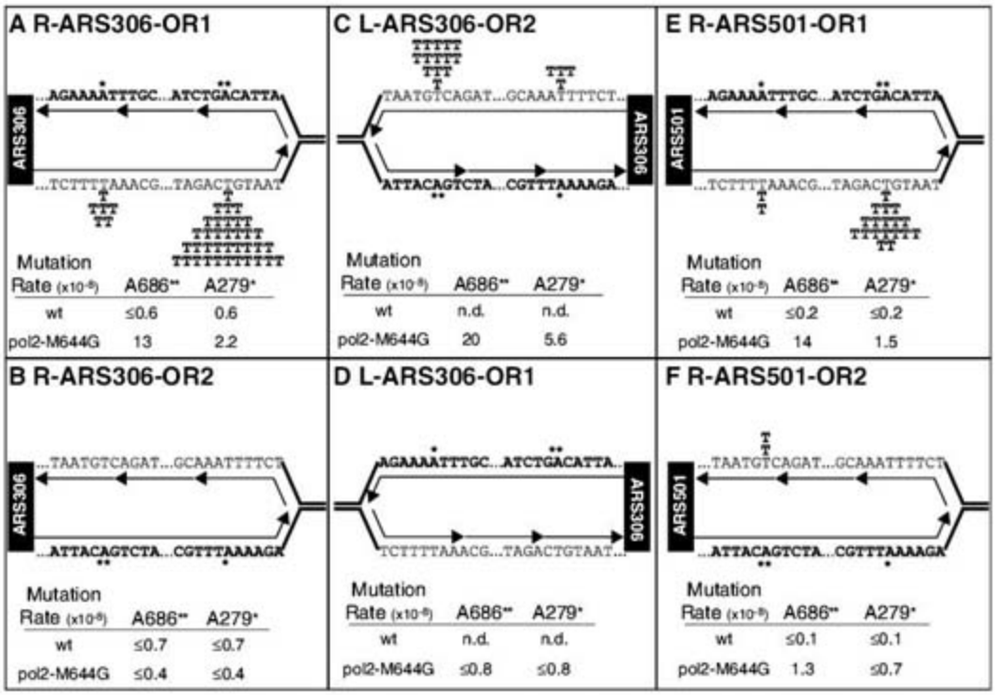

Fig. 2. Variation in the rates of T to A transversions by location and gene orientation. Six haploid strains were constructed containing the *pol2-M644G* mutant allele. *URA3* was to the right (**A**, **B**, **E**, and **F**) or left (**C** and **D**) of the indicated ARS, with the coding sequence in the Watson (A, D, and E) or Crick (B, C, and F) strand. A replication fork is shown moving away from the ARS (black box) and replicating the *URA3* gene. The nascent leading strand is depicted as a single, unbroken arrow, whereas nascent Okazaki fragments on the lagging strand are depicted as broken arrows. The A–T to T–A hot spots (* for A279 and ** for A686) are represented as the inferred T-dTMP mispair generated during replication.

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Supporting Online Material

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Materials and Methods
SOM Text
Fig. S1
Tables S1 to S3
References

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Host Resistance to Lung Infection Mediated by Major Vault Protein in Epithelial Cells

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The airway epithelium plays an essential role in innate immunity to lung pathogens. Ribonucleoprotein particles primarily composed of major vault protein (MVP) are highly expressed in cells that encounter xenobiotics. However, a clear biologic function for MVP is not established. We report here that MVP is rapidly recruited to lipid rafts when human lung epithelial cells are infected with *Pseudomonas aeruginosa*, and maximal recruitment is dependent on bacterial binding to the cystic fibrosis transmembrane conductance regulator. MVP was also essential for optimal epithelial cell internalization and clearance of *P. aeruginosa*. These results suggest that MVP makes a substantial contribution to epithelial cell–mediated resistance to infection.

The innate immune response to foreign pathogens involves a rapidly initiated, complex array of cellular changes and activation of signaling pathways after contact of pathogenic microbes with epithelial surfaces. Cellular uptake of pathogens, induction of an inflammatory cascade, and eventual apoptosis of cells to restore tissue homeostasis are essential for the clearance of many pathogens (*1*, *2*). One setting that allows for an in-depth study of innate immunity in the airway is the condition cystic fibrosis (CF), in which hypersusceptibility to infection with *Pseudomonas aeruginosa* results partly from a failure to properly activate the normal host immune response (*3*–*5*).

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Different mutations in the CF transmembrane conductance regulator (*CFTR*) gene have been found, many resulting in a deficiency in the innate immune response to *P. aeruginosa* (*6*–*8*). Most CF patients (>80%) develop chronic *P. aeruginosa* lung infections, with respiratory function decline over 10 to 25 years, and premature mortality in the third to fourth decade of life (*3*, *6*). Among its other functions, CFTR acts as a receptor for the outer core oligosaccharide of the *P. aeruginosa* lipopolysaccharide (LPS) (*5*). In humans with WT-CFTR, rapid binding to *P. aeruginosa* initiates an innate immune response that includes bacterial ingestion by epithelial cells, nuclear factor κB (NF-κB) activation, cytokine secretion, and eventual epithelial cell apoptosis (*8*–*11*). These responses are associated with maximal resistance to *P. aeruginosa* infection. In CF, the absence of functional CFTR prevents these rapid innate immune responses to infection, resulting in failure to clear *P. aeruginosa*. Infection is further exacerbated by additional features of CF, including poor mucociliary clearance due to dehydrated airway surface liquid and thickened mucus (*6*), as well as the propensity of the organism to grow as mucoid microcolonies within anaerobic mucus plugs (*12*).

P. aeruginosa infection initiates effective innate immune responses after contact with lung epithelial cells by inducing formation of lipid rafts and/or caveolae membrane microdomains (*13*, *14*) that contain WT-CFTR (*14*). To determine the molecular events that ensue from this initial epithelial cell contact, we performed an analysis of proteins recruited to lipid rafts generated during 15 min of infection with *P. aeruginosa*. Rafts were isolated from WT-CFTR–expressing human lung epithelial cell lysates, and proteins in these rafts were compared with proteins in rafts from uninfected cells (*15*). About 150 proteins identified by matrix-assisted laser desorption/ionization–time-of-flight (MALDI-TOF) mass spectrometry were found exclusively in lipid rafts of *P. aeruginosa*–infected cells (table S1), potentially having an impact on the various cellular responses that follow CFTR binding of *P. aeruginosa*. We identified one of these proteins with an abundant number of detected peptides as the major vault protein (MVP), also referred to as the lung resistance–related protein (*16*).

MVP is highly expressed in lung and intestinal epithelia, dendritic cells, and macrophages, and multiple copies of MVP assemble into 13-megadalton barrel-like ribonucleoprotein complexes known as vaults that also contain two minor proteins [vault poly(ADP-ribose) polymerase (vPARP) and telomerase-associated protein 1 (TEP1)] and untranslated vault RNA sequences (vRNAs) (*17*). Although vaults have been proposed to have a role in drug resistance, nucleocytoplasmic transport, and regulation of signaling (*18*–*24*), a definitive function for MVP or vaults has yet to be assigned as MVP knockout mice ($MVP^{-/-}$) do not have phenotypes consistent with these in vitro observations (*17*, *25*).

Western blot confirmed the rapid recruitment of MVP to lipid rafts during *P. aeruginosa* infection (Fig. 1A). Fifteen minutes after infection, the proportion of total cellular MVP in rafts isolated from airway epithelial cells expressing WT-CFTR increased dramatically, whereas the proportion of total cellular MVP recruited to rafts in *P. aeruginosa*–infected isogenic airway cells expressing only the ΔF508 CFTR protein increased to only 30% of the levels of WT-CFTR cells (Fig. 1A). Total MVP expression was identical in the two cell lines, and 10 to 15% of the total cellular MVP protein was found in rafts

of infected WT-CFTR cells. Of the minor vault components, vPARP was detected by immunoblot and vRNA was detected by reverse transcription polymerase chain reaction (RT-PCR) in raft fractions from *P. aeruginosa*–infected cells (fig. S1). Although TEP1 was not detected in the raft fractions of infected cells, this protein was readily detected in cell lysates (fig. S1). Because TEP1 is the vault component that binds vRNA, the finding of vRNA in the raft fractions suggested that TEP1 was also present at undetectable levels and that the complete vault structure was recruited to lipid rafts after infection. Green fluorescent protein (GFP)–tagged WT-CFTR was stably transfected into the IB3-1 CF cell line (ΔF508/W1282X *CFTR* alleles), and immunofluorescence staining of *P. aeruginosa*–infected cells showed colocalization of bacteria, MVP, and CFTR (Fig. 1B), which indicated that all three components were recruited to the same cellular site during infection. However, coimmunoprecipitation experiments using lysates from infected cells failed to detect a stable interaction between CFTR and MVP. Thus, it appears that the presence of WT-CFTR augments MVP recruitment to membrane microdomains in response to *P. aeruginosa* infection without direct physical association.

Disruption of lipid rafts by cholesterol extraction using methyl-β-cyclodextrin (Fig. 1C) or solubilization of rafts using octyl-D-glucoside (fig. S2) markedly reduced the level of MVP in the raft fractions. Multiple strains of *P. aeruginosa*, including strain PA01-V; two early isolates from the lungs of a CF patient (N6, N8); and an isolate from a corneal infection (6294) induced recruitment of MVP to rafts (Fig. 1D). *P. aeruginosa* strains that express a truncated form of LPS, lacking a full outer core [mutations in the *galU* or *algC* genes (*5*, *10*)] and thus incapable of binding to CFTR, did not induce raft localization of MVP in cells with WT-CFTR (Fig. 1E). This result indicates that binding of *P. aeruginosa* LPS outer-core oligosaccharide to CFTR is required for recruitment of MVP to lipid rafts.

Previously characterized CFTR-dependent epithelial cell responses to *P. aeruginosa* include bacterial uptake, NF-κB activation, interleukin 8 (IL-8) secretion, and induction of apoptosis (*4*, *8*, *10*, *11*, *26*). To determine the potential role of MVP in these responses to *P. aeruginosa*, we reduced MVP cellular expression by more than 90% using MVP-specific small interfering RNA (siRNA) (Fig. 2A). Control cells were transfected with a scrambled siRNA sequence. MVP knockdown decreased recruitment of this protein to lipid rafts 15 min after *P. aeruginosa* infection (Fig. 2B). Internalization of four different *P. aeruginosa* strains was consequently reduced to 35 to 50% of the levels seen with control cells (Fig. 2C). MVP knockdown had no effect on NF-κB activation, IL-8 secretion, or apoptosis induced by *P. aeruginosa* (fig. S3, A to C). Thus, of these four components of the innate immune response, MVP appears to be involved only in the bacterial uptake pathway. It is also possible that, due to the high expression levels of MVP in airway cells, the residual 5 to 10% of MVP present after siRNA treatment was sufficient for these other signaling and response pathways or that other proteins could substitute for MVP in these responses.

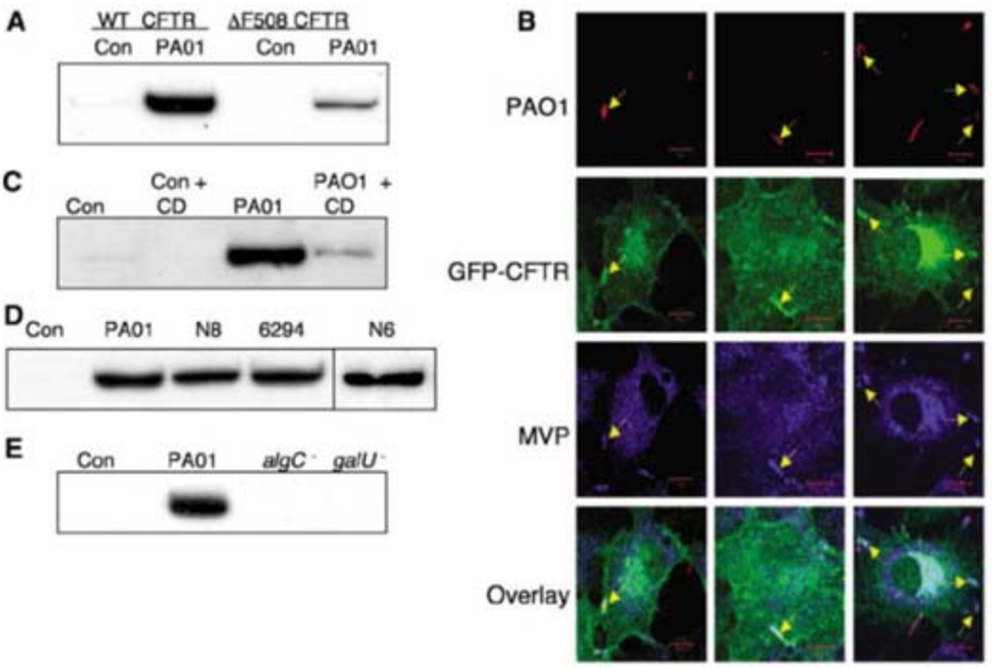

Fig. 1. Recruitment of MVP to lipid rafts of airway epithelial cells by *P. aeruginosa*. (**A**) Lysates of WT and homozygous ΔF508 CFTR human airway epithelial cells (CFT1-LCFSN and CFT1-LC3, respectively) left uninfected (Con) or infected with *P. aeruginosa* strain PA01-V (PA01) were separated on discontinuous sucrose gradients. Proteins in raft fractions were precipitated and subjected to SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot analysis with antibodies directed against human MVP. (**B**) IB3-1 CF cells (ΔF508/W1282X) transfected with WT-CFTR with an N-terminal GFP tag were infected with CFP-expressing *P. aeruginosa*, then fixed and stained for MVP. Confocal microscopy identified (arrows) bacteria (red), CFTR (green), and MVP (blue) at the site of bacterial contact with the cell membrane. Scale bar, 10 μm. Overlay shows the merged image of the three individual channels. (**C**) MVP in rafts of WT-CFTR cells left uninfected (Con) or infected with *P. aeruginosa* (PA01) in the presence or absence of 5 mM cyclodextrin (CD). (**D**) MVP in rafts of WT-CFTR cells left uninfected or infected with strain PA01-V or clinical isolates of *P. aeruginosa* from two CF patients (N6, N8) or from a corneal infection (6294). (**E**) MVP in rafts of WT-CFTR cells left uninfected or infected with strain PA01-V or LPS mutants of PA01-V (*algC*⁻ or *galU*⁻). The LPS mutants lack the CFTR-binding domain on the bacterial cell surface and do not promote MVP entry into lipid rafts after *P. aeruginosa* infection.

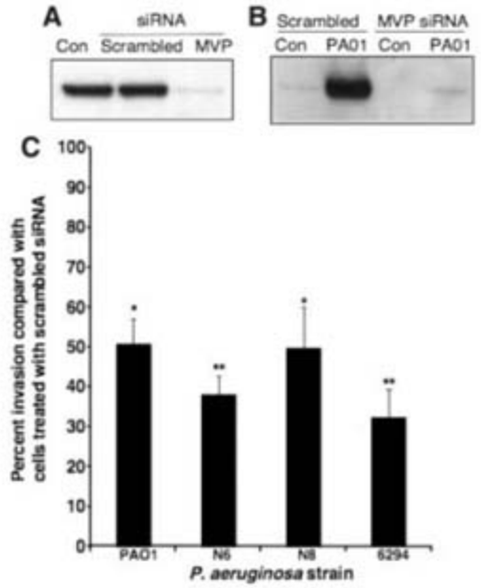

Fig. 2. MVP function in the interaction of airway epithelial cells with *P. aeruginosa*. (**A**) Whole-cell lysates from WT-CFTR cells (CFT1-LCFSN) that had been treated with either a scrambled siRNA sequence or MVP-specific siRNA were subjected to electrophoresis and immunoblot analysis with MVP-specific antibodies. Specific siRNA treatment reduced the level of MVP by >90%. (**B**) WT-CFTR cells were treated with scrambled or MVP-specific siRNA then left uninfected (Con) or infected for 15 min with *P. aeruginosa* PA01-V (PA01). Cell lysates were separated on discontinuous sucrose gradients. Raft fractions were precipitated and subjected to immunoblot analysis with MVP-specific antibodies. (**C**) Scrambled or MVP-specific siRNA-treated WT-CFTR cells were incubated with the indicated *P. aeruginosa* strains, and the amount of bacterial internalization was determined. The internalization of *P. aeruginosa* by cells treated with MVP-specific siRNA is plotted as a percentage of that obtained for scrambled siRNA-treated cells. Error bars represent SEMs. $^{*}P < 0.05$, $^{**}P < 0.01$ by a two-tailed *t* test comparison with internalization by scrambled siRNA-treated cells.

Proteomic analysis of raft proteins recovered from control or MVP siRNA-treated cells showed that MVP knockdown reduced the levels of other proteins recruited to the membrane microdomains after *P. aeruginosa* infection (table S2). The majority of the proteins reduced in infected, MVP siRNA-treated cells are involved in raft formation or cell signaling processes, consistent with the conclusion that MVP is critical for formation of stable membrane microdomains and/or raft recruitment of cell signaling molecules after *P. aeruginosa* infection. Compared with rafts from *P. aeruginosa*–infected control cells, rafts from *P. aeruginosa*–infected MVP knockdown cells had increased levels of several myosin isoforms and tropomyosin 3 (table S3).

To establish an in vivo role for MVP in host resistance to *P. aeruginosa*, we infected WT and $MVP^{-/-}$ mice intranasally with ~1.5×10^7 *P. aeruginosa* strain PA01-V and removed the lungs after 6 hours to measure total and internalized bacteria. Similar experiments using transgenic CF mice have shown that lack of CFTR results in decreased cellular internalization of bacteria and an increase in total bacteria in the lungs (*9*). Six hours after infection, virtually no innate immune cells, such as polymorphonuclear leukocytes or macrophages, were found to be associated with bacteria in WT or $MVP^{-/-}$ mice, but they were found associated with bacteria 24 hours post infection (fig. S4). Bacteria could be detected 6 hours post infection associated with epithelial cells expressing epidermal growth factor receptor (fig. S4), which indicated that bacterial internalization was primarily due to uptake by the epithelial cells.

Compared with the level of bacteria internalized by epithelial cells in WT mice, lung epithelial cells in $MVP^{-/-}$ mice internalized 55% less of the total bacteria in their lungs (Fig. 3A). This deficiency in uptake resulted in a 3.5-fold increase in bacterial burden in the lungs of $MVP^{-/-}$ mice compared with WT mice (Fig. 3B), which indicated a role for MVP in *P. aeruginosa* ingestion and clearance from the lung. MVP loss resulted in increased mortality from *P. aeruginosa* infection by a factor of almost 3 [Fig. 3C; hazard ratio = 2.96, 95% confidence interval (CI) 1.27 to 13.1; $P = 0.018$). Thus, MVP contributes to resistance against *P. aeruginosa* lung infection; this conclusion is consistent with the findings that CFTR-dependent recruitment of MVP to rafts after *P. aeruginosa* infection facilitates optimal innate immune responses to this pathogen.

Bacterial internalization by respiratory epithelial cells is an important initial step in clearance of *P. aeruginosa* (*5*, *8*, *9*) and numerous other microbes with a propensity to cause pneumonia (*27*–*29*), which indicates that uptake of bacteria by these cells enhances resistance to infection. Although the lack of MVP could cause increased susceptibility to infection through other means, the fact that the $MVP^{-/-}$ mice have normal dendritic cell function (*30*) suggests that the general phagocytic cell response would be unaltered in these mice, and that differences in outcomes would likely stem from an inappropriate epithelial cell response. We have identified a role for MVP in the response to lung infection and have validated this phenotype in $MVP^{-/-}$ mice. MVP, the predominant component of the vault complex, has been suggested to be a scaffold protein in signaling pathways and to be involved in intracellular transport (*18*, *19*, *23*, *24*), and its recruitment to lipid rafts is likely required to facilitate incorporation into rafts of additional proteins essential for responses to infection. Alternatively, MVP may stabilize or internalize the components of bacterial-induced lipid rafts by linking raft proteins to cytoskeletal elements such as tubulin or actin (*31*) or via raft-independent effects on innate immunity mediated by another function of MVP, such as the regulation of extracellular signal–regulated kinase (ERK) signaling (*24*).

There is great interest in finding human genes with nucleotide polymorphisms that modify innate immune responses, including those involved in CF lung disease (*7*). Identification and association of MVP with the cellular responses to pathogens such as *P. aeruginosa* suggests that investigation of sequence variations in the *mvp* gene or possibly other genes encoding vault components could help to define additional roles for MVP-containing structures in innate immunity.

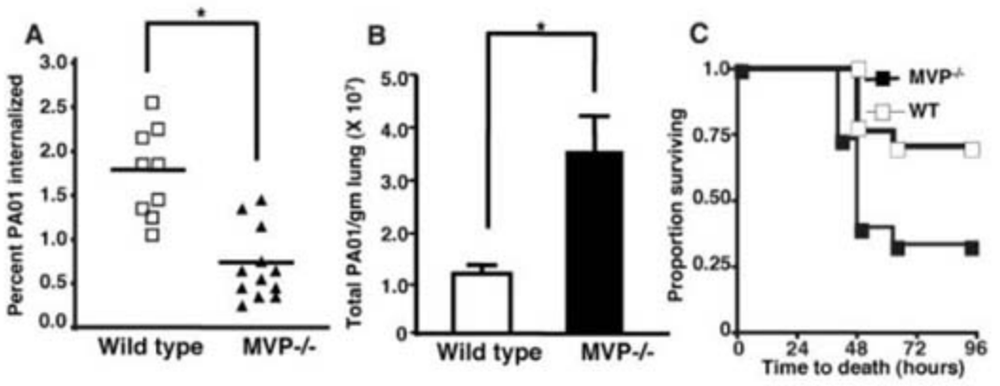

Fig. 3. In vivo role of MVP in bacterial internalization, clearance and resistance to lethality. (**A**) Six hours after intranasal inoculation with ~1.5×10^7 *P. aeruginosa* strain PA01-V, WT and $MVP^{-/-}$ mice were killed (n = 9 WT, 12 $MVP^{-/-}$), and the lungs were removed and dissociated. The internalized percentage of total bacteria found in the lung is plotted. Bars represent mean values. *$P < 0.0001$ by unpaired two-tailed t test. (**B**) The lung suspensions from the same mice used in (A) were analyzed for total bacterial load. The number of bacteria per gram of lung is plotted. Error bars represent standard error of the mean. *$P < 0.01$ by unpaired two-tailed t test. (**C**) Survival curves over 96 hours after intranasal inoculation with ~4×10^6 colony-forming units of *P. aeruginosa* strain PA01-V (n = 17 WT, 15 $MVP^{-/-}$). Hazard ratio = 2.96, 95% CI 1.27 to 13.1; $P = 0.018$.

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32. This work was funded by NIH grants R01 HL 58398-08 (G.B.P.) and R37 HL 32854-22 (D.E.G.) and by the EC FP6 RIBOREG Project (LSHG-CT-2003503022) from the European Commission (EACW). We thank A. Koh and G. Priebe for assistance with mouse studies.

Supporting Online Material

www.sciencemag.org/cgi/content/full/317/5834/130/DC1
Materials and Methods
Figs. S1 to S4
Tables S1 to S3
References

8 March 2007; accepted 5 June 2007
10.1126/science.1142311

Spectrophotometer

The NanoDrop ND-8000 8-Sample Spectrophotometer features patented technology that allows samples as small as 1 μl to be measured without cuvettes or capillaries. The system can measure even highly concentrated samples without performing dilutions. The system can process a 96-well microplate in five minutes. Up to eight samples are loaded using an eight-channel pipettor. Each sample is assessed at two different path lengths to achieve an extensive dynamic range. Total sample-to-sample measurement cycle time is less than 30 seconds, including sample prep and loading, spectral reading, and wiping of the optical surfaces. The instrument is based on a unique cuvetteless technology that allows measurement of one microliter samples pipetted directly onto the fiber optic measurement surface. Once the upper surface contacts the sample, a liquid bridge forms and a spectral reading is taken. Cleaning simply requires wiping the sample off the pedestals with an ordinary lab wipe.

NanoDrop Technologies For information 302-479-7707 www.nanodrop.com

Patent Search Software

Matheo Patent searches, retrieves, and analyzes patent data from the U.S. Patent and Trademark Office and the European Patent Office's Esp@cenet services. This software has a wide range of uses for patent searching and exploration, innovation detection, competitor and technology monitoring, patent survey, business evaluation, competitive intelligence, and more. It automatically retrieves patents based on request criteria, creates and updates the user's local database, incorporates a user classification system, defines a personalized sorting method by defining specific fields, statistically analyzes the patents collected, manages patent families, generates graphic displays, creates personal reports, and more. Pulling information from the premier European and U.S. database, the software keeps users up-to-date with automatic e-mail alerts of new pending and approved patents.

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+33 (0)4 91 08 28 82 www.matheo-software.com

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Fifteen new filter sets for fluorescent proteins include filter sets for Invitrogen's Vivid colors, Clontech's Living Colors, and MBL's Coral Hues. Because live cell experiments are typically light-starved, optimized filter sets must deliver high signal-to-noise ratio. These filter sets are manufactured using proprietary technology that produces steep slopes and accurate band placement, for maximizing excitation and emission energy and for minimizing background. With the addition of these new sets, Omega now has optimized sets for a total of 35 proteins, including proteins from the reef coral Anthozoa and the jellyfish Aequoria victoria.

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The MEL cell lines are licensed, low passage, human embryonic stem cells. Under license from the Australian National Health and Medical Research Council, the cell lines have been extensively tested with Millipore's HES-cGRO media for human embryonic stem cell culture. They are provided at early passage for maximizing the stable lifespan of the cell line and ensuring extended research time in a stable, pluripotent state. They grow as well-defined colonies, with compact cells displaying high nuclear to cytoplasmic ratios and prominent nucleoli.

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800-548-7853 www.millipore.com

Literature

The Vector Laboratories 2007–2008 catalog contains several new products plus more than 300 monoclonal and polyclonal primary antibodies and probes. New products include the ImmPRESS Peroxidase Micropolymer Immunohistochemical Anti-Goat Ig Staining Kit. Designed for detection of goat immunoglobulin G primary antibodies, this reagent provides high sensitivity, low background, and shortened staining times. The Antigen Unmasking Solution, high pH, is a TRIS-based formula highly effective at revealing antigens in formalin-fixed, paraffin-embedded tissue sections when used in combination with a high-temperature treatment procedure. Other new products include Animal-Free Blocker for blotting applications, DNA Molecular Weight Markers, and Photoprobe Amine labeling reagent that incorporates primary amines into nucleic acids. The Resolve-It Kit contains two ligands, AT-Yellow and GC-Red, that allow the separation of DNA fragments that are identical in size but contain different sequences.

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The ViraDuctin Lentivirus Transduction Kit is a novel reagent system optimized to provide superior lentivirus transduction efficiencies in a variety of cell lines. Efficiencies are typically two-fold to six-fold higher than with transductions using Polybrene, according to the manufacturer. The user-friendly ViraDuctin System can be used with non-permissive cells, including primary cells and stem cells.

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POSITIONS OPEN

PROFESSOR in RESIDENCE

The University of California, San Francisco (UCSF) Cancer Research Institute is recruiting for a Professor-level scientist to work on the role of the transforming growth factor (TGF)-beta signaling pathway in the development of cancer and related diseases. The successful candidate is expected to contribute to the understanding of this critical pathway through analysis of genetic model systems, cell biology, and molecular characterization of the components of the pathway. Consistent with the focus of the Center on translational research, this research program is expected to involve therapeutic approaches based on this pathway. The successful candidate is expected to be an active participant in the Cell Cycling and Signaling Program within the Comprehensive Cancer Center and will interact with other basic and disease-orientated programs. Laboratory space will be provided within the new Helen Diller Family Cancer Research Building at Mission Bay. The appointment will be at the Professor level in the In-Residence series.

Appointment requirements: Ph.D. or Ph.D./M.D. in biochemistry, genetics, microbiology, or cell and molecular biology. Extensive experience with established record of skilled independent research, leadership, productivity, and widespread publication history focused on TGF-beta signaling pathway through analysis of genetic model systems, cell biology, and molecular characterization of pathway components. Demonstrated ability to effectively acquire and administer funding for a full self-supporting independent research laboratory. Capacity to encourage and support independent research to build programs, mentor trainees, and generate maximum impact. Established record of academic teaching excellence.

Please send a letter of interest, curriculum vitae, and names/contact information for three references to:

Professor in Residence M2926
Human Resources
University of California, San Francisco
Cancer Research Institute
1600 Divisadero Street, P.O. Box 1297
San Francisco, CA 94143-1297

Filing deadline is July 31, 2007.

UCSF seeks candidates whose experience, teaching, research, or community service has prepared them to contribute to our commitment to diversity and excellence. *UCSF is an Affirmative Action/Equal Opportunity Employer. The University undertakes affirmative action to assure equal employment opportunity for underutilized minorities and women, for persons with disabilities, and for covered veterans. All qualified applicants are encouraged to apply, including minorities and women.*

MEDICAL WRITER-RESPIRATORY

The Prescott Medical Communications Group (PMCG), a marketing communications company serving the pharmaceutical and biotechnology industries, has immediate openings for Scientific Writer/Editors with experience in respiratory research and therapies. Candidates must possess an advanced biomedical science degree (M.S., Ph.D., Pharm.D., M.D.) and a minimum of five years of continuing medical education or agency (or similar) experience. This full-time in-house position will require residing in the Chicago area and occasional domestic/international travel. PMCG offers an unparalleled opportunity for professional development in a fast-paced and intellectually challenging environment. Please send employment history and three writing samples to: **Jim Bachleda, Vice President of Operations, Prescott Medical Communications Group, 205 N. Michigan Avenue, Suite 3400, Chicago, IL 60601. Fax: 312-528-3901. E-mail: jbachleda@prescottmed.com.**

SK BIO-PHARMACEUTICALS

ELECTROPHYSIOLOGIST, patch clamping, ex vivo and in vivo cardiac safety (Ph.D).

Candidates with pharmaceutical industry experience desired. Qualifying candidates should forward resume/curriculum vitae with cover letter to: **Human Resources Department, SK Bio-Pharmaceuticals, 22-10 Route 208 S., Fair Lawn, NJ 07410. Fax: 201-796-2278; e-mail: elee@skbp.com.**

POSITIONS OPEN

EDITOR-IN-CHIEF

The Society for Risk Analysis (SRA) seeks nominations for Editor-in-Chief (EIC) of *Risk Analysis: An International Journal.* The EIC works with a managing editor and six area editors in ecological and environmental risk assessment, engineering, health risk assessment, microbial risk assessment, social sciences, and decision sciences. Candidates should have strong writing and managerial skills, strong technical accomplishments in one or more disciplines relevant to SRA, preferably prior experience as an editor of scholarly publications, and broad interests. More details at **website: http://www.sra.org.** For full consideration, submit statement of qualifications, relevant experience, management approach, plans and strategic vision for the journal, curriculum vitae, and a list of references by September 1, 2007, to: **Dr. H. Christopher Frey, Campus Box 7908, North Carolina State University, Raleigh, NC 27695-7908** or by **e-mail: frey@ncsu.edu.**

Indiana University, Purdue University, Indianapolis (IUPUI), invites applications and nominations for the senior academic position of **VICE CHANCELLOR for RESEARCH.** IUPUI is Indiana's leading urban public research university with about 30,000 students and over 1,300 tenure/tenure-track faculty, granting undergraduate, graduate, and professional degrees in over 180 areas. The Vice Chancellor for Research is responsible for policy making and oversight responsibility for the research mission of the IUPUI campus. The position requires an individual with a broad vision who is able to integrate research programs in Indianapolis and encourage opportunities for collaboration with other campuses of Indiana University and Purdue University, as well as the not-for-profit and for-profit sectors. External grant funding at IUPUI exceeded $261 million last year. More information about the position can be found at **website: http://iupui.edu/vcresearchsearch/.** Candidates should have a Doctorate or equivalent terminal degree and understanding of research funding. Applications and nominations will be accepted through September 17, 2007. Submit a statement of vision and philosophy for strengthening IUPUI's research enterprise, a portfolio of past experiences, curriculum vitae, and six professional references to:

H. Öner Yurtseven, Chair
Vice Chancellor for Research Search Committee
c/o Susan Martin (e-mail: sujmarti@iupui.edu)
School of Engineering and Technology
Indiana University, Purdue University, Indianapolis
799 W. Michigan Street, ET331F
Indianapolis, IN 46202

Online application is also available at **website: https://www.et.iupui.edu/apply/index.asp?pos=IN-ENGT07004.** *IUPUI is an Affirmative Action/Equal Opportunity Employer, Educator, and Contractor, Minorities/Females/Persons with Disabilities.*

STAFF BIOCHEMIST

Perform electrophysiological studies to determine effects of novel drugs on spinal cord neuron activity following inflammation or neuropathy. Evaluate analgesic activity of novel drugs for inflammatory and neuropathic pain. Design experiments, collect, analyze, and summarize data, using various math and statistical software/program languages. Minimum requirements: M.S. in biology, chemical engineering, or biomedical engineering and two years of laboratory experience performing electrophysiological and behavioral studies with rodents. Job location: West Point, Pennsylvania. Curriculum vitae to: **J. Kennedy, Merck Research Laboratories, WP1-3, 770 Sumneytown Pike, P.O. Box 4, West Point, PA 19486.** *Equal Opportunity Employer.*

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Positions @ NIH

THE NATIONAL INSTITUTES OF HEALTH

NIDDK

Program Director in Gestational Diabetes and Obesity Research
Health Scientist Administrator, GS-14

The Division of Diabetes, Endocrinology and Metabolic Diseases (DDEMD), National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institutes of Health (NIH) is expanding its programs in obesity and diabetes research. An opportunity exists for a clinical scientist to join a dedicated and dynamic group of health scientist administrators to help guide NIH funding of research in diabetes and obesity.

A clinically-oriented scientist with expertise in the areas of obesity and gestational diabetes is sought for this new position. This individual will be responsible for managing and developing a program of research grants in the human physiology of gestational diabetes, imprinting and obesity. A person with an interest in fostering basic and clinical partnerships is desirable.

This position involves close interaction with leading researchers, scientific administration of grants and contracts, program planning and development, and the opportunity to organize and attend scientific meetings. The successful candidate will have independent research experience, with a track record of publications in areas of biomedical science relevant to the objectives of the program, excellent interpersonal and written communications skills, the ability to identify research priorities and opportunities, and the ability to track and analyze the success of initiatives and programs. In carrying out these responsibilities, the Program Director will interact with national leaders in diabetes, endocrine, obstetric, and obesity research. Many DDEMD research activities are conducted through partnerships between the NIDDK and other components of NIH and DHHS, as well as voluntary organizations. The Program Director will play a leadership role in fostering these partnerships.

This position is subject to a background investigation. Applicants must be U.S. citizens, resident aliens, or nonresident aliens with an employment-authorized visa, and have an advanced degree (M.D., Ph.D. or equivalent) along with relevant independent clinical and/or basic research. The position is located in Bethesda, Maryland. Salary and benefits will be commensurate with the experience of the applicant.

Position requirements and detailed application procedures are provided on Vacancy Announcement Numbers: **NIDDK-07-193870-DE, and NIDDK-07-193871-MP**, which can be obtained by accessing **WWW.USAJOBS.GOV**. All applications must be received by **07/31/2007**. For additional information contact **Karen Page at (301) 496-4232**.

NIDDK

HEALTH SCIENCE POLICY ANALYST
(Two Positions Available)

The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) is seeking applications from individuals who are currently in post-doctoral positions in biomedical research laboratories, but who wish to make a career change from a laboratory setting. Particularly encouraged to apply are individuals with post-doctoral experience in molecular biology, coupled with demonstrated writing and other communication skills. Incumbent will develop a wide range of documents that analyze and present the scientific accomplishments and plans of the NIDDK to public policy makers, voluntary health organizations, and other lay audiences. Incumbent must thus be able to convey in understandable, scientifically accurate, and meaningful terms the contributions of biomedical research to human health. Total salary is competitive and will be commensurate with the experience of the selectee.

Position requirements and detailed application procedures are provided on Vacancy Announcement Numbers: **NIDDK-07-197281-DE and NIDDK-07-197281-MP**, which can be obtained by accessing **WWW.USAJOBS.GOV**. All applications must be received by **07/20/07**. For additional information contact **Karen Page, Human Resources Specialist at (301) 496-4232**.

NATIONAL CANCER INSTITUTE

Postdoctoral Position

The Biomolecular Structure Section (BSS) of the Macromolecular Crystallography Laboratory, National Cancer Institute at Frederick is inviting applications for a postdoctoral position. The appointment will be two years initially and renewable up to a total of 3-5 years on the basis of performance and mutual agreement. Qualified applicants should have experience in macromolecular crystallography and skills in either insect cell protein expression or structure-based drug design. Successful candidate will participate in the structure/activity studies of RNA-processing proteins or structure-based development of anticancer and antimicrobial agents. Additional information about research activities in the BSS can be found at: **http://mcl1.ncifcrf.gov/ji.html**. We provide excellent work environment, state-of-the-art facilities and regular access to synchrotron light source; we offer competitive salary and comprehensive health insurance. DHHS and NIH are Equal Opportunity Employers. Please send CV, research summary, and contact information of three references to **Dr. Xinhua Ji at: jix@ncifcrf.gov**.

WWW.NIH.GOV

National Center for Complementary and Alternative Medicine
HEALTH SCIENTIST ADMINISTRATORS

The National Center for Complementary and Alternative Medicine (NCCAM), a component of the National Institutes of Health (NIH) in the Department of Health and Human Services (DHHS), is seeking applications from exceptional scientists to serve as **Health Scientist Administrators (HSAs)** in its Division of Extramural Research and Training. As an HSA, you will develop a strategic vision for complementary and alternative medicine research; implement plans to achieve that vision; provide stewardship of awarded grants and contracts; and serve as a scientific expert within your field.

Qualifications: The successful individuals will possess a Ph.D., M.D., or equivalent degree and a record of research accomplishment in the areas of neuroscience, molecular biology, biochemistry, chemistry, pharmacology, and/or clinical research; the successful candidates are also expected to demonstrate strategic vision; excellent communication skills; and the ability to work collaboratively in a team.

Salary: The current salary range is $79,397.00 - $121,967.00, depending on experience and accomplishments; a full Civil Service package of benefits (including retirement, health, life and long-term care insurance, Thrift Savings Plan participation, etc.) is available. Recruitment incentive may be awarded and relocation expenses may be paid.

How To Apply: Position requirements and detailed application procedures are provided in vacancy announcements **NCCAM-07-198702-CR-DE**, which can be found at **http://nccam.nih.gov.** All applications and supplemental information must be received no later than **Friday, August 10, 2007**. For additional information, contact **Ms. Kathy Delauter at 301-594-2283 or delauterk@mail.nih.gov**.

Staff Scientist
Laboratory of Infectious Diseases
Respiratory Viruses Section

The National Institute of Allergy and Infectious Diseases, a major research component of the NIH and the Department of Health and Human Services, is recruiting a Staff Scientist. The position will be available in the Respiratory Viruses Section of the Laboratory of Infectious Diseases, and scientists with a M.D. or Ph.D. are eligible. The research activity involves (1) the development of live attenuated flavivirus vaccine candidates and their evaluation in rodents and non-human primates as well as in the clinical trials in humans; (2) the use of novel approaches for construction of chimeric viruses to examine basic questions of viral pathogenesis and the molecular basis of attenuation of highly neurovirulent flaviviruses; (3) the evaluation of the immunologic determinants of resistance to infection and illness caused by these flaviviruses. This full-time research position offers a unique opportunity to work on investigations that range from basic molecular biology to applied vaccinology. Staff Scientist applicants should have at least six years of laboratory work experience in molecular virology and immunology; the salary range is $74,503 - $162,371. Preference will be given to candidates who have experience working with neurotropic viruses. Applicants should submit their curriculum vitae, a letter of research interests, and names and addresses of three references to:

Alexander Pletnev, NIH/NIAID/LID, 33 North Drive, Room 3W10A, Bethesda, MD 20892-3203, FAX: (301) 496-0501, email: apletnev@niaid.nih.gov. Review of applicants will begin on **September 1, 2007** and continue until a successful candidate is identified.

Staff Scientist
Laboratory of Infectious Diseases
Respiratory Viruses Section

The National Institute of Allergy and Infectious Diseases, a major research component of the NIH and the Department of Health and Human Services, is recruiting a Staff Scientist. The position will be available in the Respiratory Viruses Section of the Laboratory of Infectious Diseases, and scientists with a M.D., D.V.M, or Ph.D. are eligible. The research activity involves (1) examination of the pathogenesis of pandemic and potential pandemic strains of influenza and their evaluation in vitro and in experimental animals including the 1918 influenza; (2) influenza viral genomics, and examination of viral evolution in fitness and host adaptation; and (3) the development of influenza clinical trials in humans. This full-time research position offers a unique opportunity to work on investigations that range from basic molecular biology to clinical research. Staff Scientist applicants should have at least six years of laboratory work experience in molecular and classical virology research; the salary range is $74,503 - $162,371. Preference will be given to candidates who have experience working with influenza viruses especially those with BSL3 experience. Applicants should submit their curriculum vitae, a letter of research interests, and names and addresses of three references to:

Jeffery K. Taubenberger, MD, PhD, Attn: A. LeCointe, NIH/NIAID/LID, Bldg 33/Room 3W02B, MSC 3203, 33 North Drive, Bethesda, MD 20892-3203, FAX: (301) 480-4509, email: lecointe@niaid.nih.gov

Review of applicants will begin on **July 2, 2007** and continue until a successful candidate is identified.

Argonne
NATIONAL
LABORATORY

The X-ray Science Division at Argonne National Laboratory invites applications for staff positions within X-ray Operations and Research. We are seeking candidates in all experimental x-ray areas, with particular emphasis on inelastic x-ray scattering, magnetism, small-angle x-ray scattering, surface scattering, and x-ray imaging. Positions are entry level to senior appointments.

Candidates should have a strong background in synchrotron radiation research; considerable skill in developing instrumentation (such as x-ray optics and detectors) and in state-of-the-art techniques; considerable skill in designing and implementing x-ray optics; considerable skill in understanding abstract concepts, experience in hardware and software for computer control/data collection systems and high heat load x-ray optics is beneficial.

The Advanced Photon Source at Argonne provides a stimulating intellectual environment, and offers the successful candidates many opportunities to interact with world-class facilities and researchers.

Successful candidates will have a Ph.D., postdoctoral experience and a demonstrated ability to conduct high-quality independent research in the field.

Argonne is a U.S. Department of Energy laboratory managed by UChicago Argonne, LLC. Argonne's site is approximately 25 miles southwest of Chicago on a beautiful 1500-acre campus-like environment. Interested candidates should send a detailed CV by August 24, 2007, along with a list of publications, and the names and addresses of three references through the Argonne website at **http://www.anl.gov/jobs** job search for the following requisitions:

XSD 311367 (Assistant Physicist)
XSD 311262 (Physicist)
XSD 311364 (Physicist)

For additional technical information, please contact Dr. George Srajer at **XSDpositions@aps.anl.gov**.

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PROFESSOR POSITION

Department of Environmental and Occupational Health

Graduate School Public Health
University of Pittsburgh

The University of Pittsburgh is holding a search for a non-tenure faculty in the area of environmental oncology/environmental genomics. The faculty position is at the professor level. Successful candidates will have an outstanding record of research in genomic environmental oncology and a strong interest to help translate these discoveries to preventive public health strategies. The faculty would be expected to interact with the established groups within the Department of Environmental and Occupational Health, Graduate School of Public Health as well as within The Center for Environmental Oncology at the University of Pittsburgh Cancer Institute focused on molecular carcinogenesis, toxicogenomics and DNA repair. The candidate is expected to have an established research program and proven ability to develop and manage interdisciplinary research projects. We seek a candidate with vision, passion and energy, who can move our environmental oncology/genomics program into greater prominence in ongoing and forthcoming funding opportunities at national and international levels. In addition, the candidate will be expected to teach environmental genomics at PhD, MS/MPH levels.

Applications will be received until position is filled. Interested applicants should send letter of intent, a detailed curriculum vitae, a 1-2 page description of research and teaching objectives, and contact information for three references:

Chair, Search Committee on Environmental Oncology/Genomics
Department of Environmental and Occupational Health
Bridgeside Pointe
100 Technology Drive, Rm 328A
Pittsburgh PA 15219
recruitment@eoh.pitt.edu

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Tenure Track Position in Pediatric Cardiovascular Research

As part of a significant enhancement of its basic science and translational research programs, Weill Cornell Medical College is undertaking a major program initiative in the area of Pediatric Cardiovascular Research. **The Department of Pediatrics is seeking a tenure track faculty investigator to establish a research program in cardiovascular biology or related themes of investigation, including angiogenesis, developmental biology or cell biology.** We are especially interested in candidates with established research programs who will be interactive scientifically with Cornell University researchers on the New York City/Weill and Ithaca/Cornell University campuses. Candidates for intermediate-level positions should demonstrate the potential for establishing a vigorous independent research program, and candidates for senior positions should have an outstanding record of productivity. Candidates may have Ph.D., M.D., or M.D.-Ph.D. degrees. Primary appointment of tenure track faculty as part of this initiative will be in the Department of Pediatrics.

Recruited faculty will receive support from an endowment. Candidates may participate in the Weill Cornell Graduate School of Medical Sciences. In addition, candidates may participate in the Tri-Institutional MD-PhD Program which also includes faculty from The Rockefeller University. Applications should include a curriculum vitae, statement of research interests and three letters of recommendation. Applications should be sent to:

Anne Moscona, M.D., Chair/Cardiovascular Research Recruitment Committee, Box #309
Department of Pediatrics
Email: anm2047@med.cornell.edu
FAX: 212-746-8261

Weill Cornell Medical College
1300 York Avenue, New York, NY 10021
EOE/M/F/D/V

www.med.cornell.edu/jobs

West Virginia University

Associate Director for Basic Research

The Mary Babb Randolph Cancer Center (MBRCC) seeks an outstanding cancer research scientist to lead our basic research programs. The successful candidate must be a PhD or MD/PhD level scientist; have a funded track record in basic cancer research and a commitment to developing translational research programs and maintaining an excellent graduate education program in Cancer Cell Biology. We are particularly interested in researchers whose programs focus on tumor microenvironment, although we welcome applications from researchers who have evident strengths in signal transduction or molecular therapeutics research areas.

The MBRCC is the only Cancer Center in the State of West Virginia that incorporates patient care, patient research, basic research and population science into its mission. The MBRCC has a focus on patient populations in WV and Appalachia, where there are significant patient populations with Lung, Breast, GI and prostate cancer, and there exist health disparities in Lung, Head & Neck, Ovarian, Cervical cancer, as well as breast cancer mortality. Basic research strengths of the program include signal transduction and tumor microenvironment, with emerging programs in Lung (focus on nanotechnology) and developmental therapeutics.

This is an exciting time to join the MBRCC, which is currently undergoing a $21 million expansion that will nearly double clinic space and promote the development of a Phase I/II clinical trials program, a new research building that will increase laboratory space and a significant investment in technology and core facilities, and is launching a major faculty recruitment campaign. The cancer research program is fully integrated into the institutional strategic research plan with the expansion of the WVU Health Sciences Center campus and recruitment of research and clinical faculty. The cancer center is poised for tremendous growth over the next 5 years.

Morgantown consistently ranks as one of the Best Small Cities in America, is located just one hour south from Pittsburgh, Pa and three hours from Washington, DC and Baltimore, MD. It offers culturally diverse, large-city amenities in a safe, family setting.

The position will remain open until filled. A start-up package, salary and benefits will be commensurate with experience with joint appointment to the MBRCC and an appropriate academic department. Interested applicants should submit a cover letter indicating research interests, curriculum vitae and three references to: **Daniel C. Flynn, PhD, Deputy Director, MBRCC, c/o Donna Tamasco, West Virginia University, Mary Babb Randolph Cancer Center, P.O. Box 9300, Morgantown, WV 26506; Email: dtamasco@hsc.wvu.edu; Phone: (304) 293-0781.**

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Senior Vice President and Vice Provost for Research

The State University of New York (SUNY) and The Research Foundation of SUNY (RF) are seeking a Senior Vice President and Vice Provost for Research to serve as a senior member of the University's Research Foundation, located in Albany, New York. This is a newly created position that will provide leadership, vision and strategy for the administration of research and sponsored programs across the State University supported by the Research Foundation and a close linkage between the Research Foundation and the core academic mission of the University. In 2006, the Research Foundation managed more than 10,200 campus-based research projects, employing over 18,000 faculty and staff with total funding of over $895 million. They are ranked ninth in research expenditures among 208 institutions in the U.S., Europe and Canada. This individual will be tasked to serve as a member of the senior administrative team of the Office of the University Provost and, holding the designation as Vice Provost, will be respsonsible for making decisions and recommendations affecting the academic and research strength of SUNY campuses. The individual will be the primary research liaison to the university research centers. The University is in the process of doing major "cluster hiring" as part of the Empire Innovation Program, and a major task for the Vice Provost will be to monitor and enable the successful implementation of this program. In addition, the candidate, as Vice Provost, will work closely with campus administrators to recruit and retain strong graduate students in order to enable the SUNY system both to meet its overall research goals and to recruit and retain the strongest research faculty on the individual campuses.

The successful candidate will have at least 10 years of progressively more complex leadership experience in academic research management, strategy and policy development or related activity. The ideal candidate will have the ability to partner with peers and other business leaders to build consensus and implement positive change. Must have successful experience interacting with a broad array of constituents; ability to manage multiple priorities and divergent viewpoints. Must have the ability to foster an environment of teamwork, strong influencing skills. It is expected that the successful candidate will have an earned Doctorate or equivalent degree and have had experience as a faculty member in a research university setting. The individual should have held the position of full professor with teaching and research accomplishments.

Spencer Stuart has been retained to assist the Research Foundation at the State University of New York with this most important recruitment. Spencer Stuart and the Search Committee respect the importance of maintaining confidentiality. Letters of application, with curriculum vitae, and letters of nominations should be submitted by email (preferred) to: **dwestmore@spencerstuart.com.** Or by mail to: **Charles M. Falcone, MD, MBA, Spencer Stuart, 401 N. Michigan Ave., Ste. 3400, Chicago, IL 60611.**

UNIVERSITY OF OXFORD

Sir William Dunn School of Pathology

César Milstein Professorship of Cancer Cell Biology

Applications are invited for the above endowed Chair, to be held at the Sir William Dunn School of Pathology, and tenable from as soon as may be arranged. The successful candidate will have demonstrated a capacity or potential for senior academic leadership in the area of cancer cell molecular biology. He or she will be able to show evidence of management skills, an impressive publication record, and an ability to raise extensive research grant income. Applications are welcomed from scientists with a strong interest in basic mechanisms underlying cancer development and progression.

A non-stipendiary fellowship at Lincoln College is attached to the professorship.

Further particulars, including details of how to apply, are available from http://www.admin.ox.ac.uk/fp/ or from the Registrar, University Offices, Wellington Square, Oxford OX1 2JD Tel. (01865) 270200. The closing date for applications is 3rd September 2007.

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In this independent role, you will implement & oversee in vitro and ex vivo studies focused on inflammatory or immunological endpoints for a diverse set of biological targets. Develop & optimize compound enabling systems in primary immune cells that contribute to program advancement. Critically evaluate literature and in-house data in order to devise innovative approaches to problem solving and propose new targets. Lead early or late-stage programs, serve as functional representative in a program team environment & substantially contribute to team screening cascades, timelines and goals. Analyze and present high-quality data and supervise activities of at least one associate.

Ph.D. in Immunology, Cell Biology or Pharmacology with at least 2-4 years postdoctoral experience required. Must be recognized as a scientific expert in immunology, inflammation or autoimmune disease. The ability to critically appraise scientific literature in order to impact practical and strategic approaches to projects (i.e., initiate programs, disease strategies, screening cascades, etc.) is essential. Must possess knowledge of multiple components of the drug discovery process learned through participation in and leadership of early or late-stage programs. Research experience gained in the pharmaceutical industry desirable. Research experience with human primary immune cells, whole blood assays and competency in FACS-based analysis also desirable. Knowledge and experience with in vitro or ex vivo human or rodent immunocytes is essential. In-depth knowledge of immune cell functional responses, and knowledge of in vivo models a plus.

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Institute of Advanced Study
2008/09 Distinguished and Fast-Track Fellowships

The Institute of Advanced Study is a flagship project launched in October 2006 to mark the 175th anniversary of the foundation of Durham University. It offers a number of funded fellowships for a three-month period (October-December 2008 or January-March 2009). Applications are invited from world-ranking public intellectuals, academics and non-academics, in any discipline, based abroad or in the UK, and especially from individuals interested in inter-disciplinary dialogue. The theme for 2008/09 is "Being Human", interpreted in its broadest sense to be of intellectual interest to those working across the Sciences, Social Sciences, Arts and Humanities. Most, but not all, of the Fellows will be linked to this theme. Distinguished and fast-track fellowships are available. Fellowships include a stipend, travel, accommodation, subsistence and costs associated with replacement teaching (where appropriate).

Further particulars are available to download from the Institute of Advanced Study's website www.durham.ac.uk/ias. Further details of the 2008/09 Being Human theme can also be found here. Informal enquiries with the Directors of the Institute can be made via Catherine Paine, the IAS administrator (Tel: +44(0)191 3342589 or email catherine.paine@durham.ac.uk).

Closing date for applications is Friday 10th August 2007.

MAYO CLINIC

FACULTY POSITION IN CANCER RESEARCH

Rochester, MN

The Mayo Clinic College of Medicine, Division of Experimental Pathology and the Mayo Clinic Cancer Center in Rochester, MN, is conducting a search for a highly competitive scientist at the Assistant, Associate, or Full Professor level with a focus on cancer research. All areas of cancer research experience are of interest although a focus on the hematologic malignancies or breast cancer is preferred. The Mayo Clinic in Rochester provides a unique research environment that combines state-of-the-art research facilities and an exceptional academic medical center. The Mayo Clinic Cancer Center is an NCI-designated Comprehensive Cancer Center.

To learn more about Mayo Clinic or Rochester, MN please visit **www.mayoclinic.org**.

Interested applicants should submit their curriculum vitae, list of potential references and brief statement of research accomplishments, interest and future directions. Application material should be submitted electronically to **searchcomm@mayo.edu**.

George G. Klee, M.D., Ph.D.
Experimental Pathology and Laboratory Medicine, Chair
Search Committee, Chair
Professor of Laboratory Medicine and Pathology
Mayo Clinic College of Medicine

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www.sciencecareers.org

2007 INTERNATIONAL SYMPOSIUM ON PROTEIN MODIFICATION AND DEGRADATION IN BEIJING

November.4-7, 2007 Beijing International Convention Center, Beijing, China

Keynote speakers

Aaron Ciechanover

2004 Nobel Laureate in Chemistry, Professor, Technion-Israel Institute of Technology, Israel

Marc Kirschner

Professor and Chairman, Department of Systems Biology, Harvard Medical School, Boston

Tse Wen Chang

Inventor of anti-IgE therapy for asthma and allergyDistinguished Research Fellow, Genomic Research Center, Academia Sinica, Taipei

Presidents

Depei Liu

Professor and President, Chinese Academy of Medical Sciences

Zhu Chen

Professor and Vice President, Chinese Academy of Sciences, China

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Invited speakers

Aaron Ciechanover	Raymond J Deshaies	Peter Jackson	Tom Rapoport
Ning Zheng	Alfred L. Goldberg	Daniel Finley	Keiji Tanaka
George DeMartino	Kun Ping Lu	Yi Zhang	Xi He
Marc Kirschner	Michael Karin	Zhijian James Chen	Chin Ha Chung
Xingwang Deng	Stefan Jentsch	Jesper Svejstrup	Brenda Schulman
Yong Wan	Tse Wen Chang	Maria G Masucci	Ze'ev Ronai
David Rubinsztein	Xiaodong Wang	Cheng Cao	Depei Liu
Yue Xiong	Junying Yuan	Qimin Zhan	Zhu Chen
Stuart Lipton	Hugues de Thé	Allan Weissman	Kenneth Rock

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Tsinghua University, China
Karolinska Institutet, Sweden
Harvard Medical International, USA

Register before Jul. 31, 2007 to save USD 100

CGIAR

CGIAR Challenge Programs Call for Pre-Proposals

The Consultative Group on International Agricultural Research (www.cgiar.org) invites all interested parties to develop pre-proposals on any of the following ideas for Challenge Programs: (a) climate change, agriculture and food security; (b) high-value crops (fruits and vegetables); and (c) combating desertification (dryland degradation). Concept notes on the above topics are found at:

http://www.cgiar.org/impact/challenge/cp_cycle2.html

CGIAR Challenge Programs (CPs) have the following characteristics:

- Address complex issues of great global and/or regional significance
- Involve high impact research relating to CGIAR goals
- Work through broad partnerships involving a wide range of institutions
- Are independently governed
- Are committed to achieve specific objectives within an agreed time frame

Each CP involves at least two CGIAR centers and research organizations from two developing country national agricultural research systems.

Pre-proposals may be developed and submitted by any organization capable of managing a major international agricultural research program. These must be submitted via email no later than **September 10, 2007**, to the following address: **cpprepropposals@cgiar.org**.

More detailed information is available at:

http://www.cgiar.org/impact/challenge/cp_cycle2.html

LAGRANGE PRIZE – FONDAZIONE CRT On Complex Systems

Notice of Competition -Year 2007

Fondazione CRT ***(Cassa di Risparmio di Torino Foundation)***, a private non profit body founded in 1991 that operates mainly in the North-Western Italian region, intends to establish with the scientific coordination of **ISI Foundation** the **Lagrange Prize – Fondazione CRT**, dedicated to scientific research in the field of complex systems.

AIM AND CRITERIA
The **Lagrange Prize – Fondazione CRT** aims at promoting research activities on complex systems. Both theoretical and experimental research activities are taken into account. In particular, the prize will be awarded to such scientific research which, having generated a large impact on the international scientific community, has enabled the accomplishment of exceptional results in the science field of complex systems and its applications in the decade prior to the publishing of this notice of competition.

THE PRIZE
The **Lagrange Prize – Fondazione CRT** is assigned every year to two researchers. The prizes, each one endowed with the amount of **€ 75,000** (seventy-five thousand euros), are assigned to individual researchers whom will be proclaimed as winners within **December 2007** by the Scientific Commission. The prize-awarding ceremony will take place in Turin by **April 2008**.

SUBMISSION OF CANDIDACIES
Each candidacy can be submitted by at least one **guarantor**, who will have to attach a dossier motivating the specific candidacy completing it with minimum 5 - maximum 10 supporting letters from a matching number of sponsors of the candidacy itself. Guarantors or sponsors of the candidature can be considered: directors and researchers of national and international research centres; professors of national and international university institutes; partners and members of Scientific Academies.
Self-candidatures will not be accepted.

CANDIDATURE DOSSIER
The guarantor of the candidacy is required to submit the related candidature dossier drawn up in English and including the following:
1) Candidacy proposal of the guarantor.
2) A written text presenting the research made by the candidate, the importance of results achieved and an analysis of the scientific impact (theoretical or experimental) brought about by the candidate's work.
3) Candidate *curriculum vitae*.
4) List of scientific publications related to the candidate's research activities. The whole dossier has to be submitted via email to **segreteria@isi.it**. The files related to the candidacy documents will have to be in pdf or word format.
5) 5-10 letters supporting the candidacy.
The prize regulation is available on the website **www.progettolagrange.it**.

SELECTION OF CANDIDATURES
The Scientific Commission will communicate the choice of the two winners within **December 2007**.

DEADLINE FOR CANDIDACY SUBMISSION
The candidacy dossier related to the year 2007 and including all documents as per art. 4, has to be received by ISI Foundation within **October 30th, 2007**.

The complete notice of the competition is available upon request addressed to the Prize Organizing Secretary (segreteria@isi.it) and can be retrieved in electronic format by accessing the website www.progettolagrange.it.

POSITIONS OPEN

ASSISTANT PROFESSOR
Informatics for Natural Resources and Ecology
School of Natural Resources

The School of Natural Resources at the University of Arizona in Tucson invites applications for a tenure-track faculty position at the Assistant Professor level (40 percent teaching, 60 percent research; nine-month appointment) with expertise related to natural resources/ecological informatics. We seek a colleague to provide leadership and expertise in methods and applications for integration of large biological, physical, social, and economic datasets across spatial and temporal scales to support natural resource management decision making. Desired qualifications include one or more degrees in natural resources or related discipline; expertise in computer modeling of natural systems, heterogeneous data structures, and data-model integration as a framework for decision making at regional scales; expertise related to interactions among climate variability, land use and habitat change, and ecosystem structure, function and environmental services; and experience working on collaborative, multidisciplinary teams. A full description is available by referencing job #37617 online at **website: http://www.uacareertrack.com.** The positions will remain open until filled, with formal reviews beginning August 20, 2007. Applications must be submitted online. For further information, contact: **David Breshears, Chair, Informatics Search Committee** at **e-mail: daveb@cals.arizona.edu** or **website: http://www.cals.arizona.edu/srnr.**

The University of Arizona is an Affirmative Action/Equal Opportunity Employer committed to recruiting, supporting, and fostering a diverse community of outstanding faculty, staff, and students. All applicants who share this goal are encouraged to apply.

TWO TENURE-TRACK ASSISTANT PROFESSOR POSITIONS
Molecular Biology/Microbiology/Bioinformatics
California State University, Fullerton

California State University, Fullerton, Department of Biological Science invites applications for two tenure-track Assistant Professor positions to begin in August 2008. The applicants must have a Ph.D. in a field relevant to molecular biology and related postdoctoral research experience. The successful candidates will be expected to develop active, externally funded research programs related to microbiology or to bioinformatics/genomics. They will be encouraged to establish interdisciplinary collaborations and expected to involve undergraduate and Master's level students in their research. Teaching responsibilities will include introductory and advanced-level courses in microbiology, molecular biology, genetics, or bioinformatics. The successful candidate must be committed to excellence in teaching a diverse population of students. Send: (1) curriculum vitae (including a history of grant support), (2) a statement of research plans and potential for collaboration, (3) two or three related publications, (4) a statement of teaching philosophy, experience, and preferences for upper-division elective courses, and (5) three letters of recommendation to: **Chair, Molecular Biology Search, Department of Biological Science, California State University, Fullerton, P.O. Box 6850, Fullerton, CA 92834-6850 (website: http://biology.fullerton.edu).** Review of applications will begin 1 October 2007, and continue until suitable candidates are appointed. *Women and minority candidates are particularly encouraged to apply. Affirmative Action/Equal Opportunity/Title IX/ADA Employer.*

CAREER OPPORTUNITY

This unique program offers the candidate with an earned Doctorate in the life sciences the opportunity to obtain the Doctor of Optometry (O.D.) degree in 27 months (beginning in March of each year). Employment opportunities exist in research, education, industry, and private practice. Contact the **Admissions Office, telephone: 800-824-5526** at the **New England College of Optometry, 424 Beacon Street, Boston, MA 02115.** Additional information at **website: http://www.neco.edu, e-mail: admissions@neco.edu.**

POSITIONS OPEN

INSTITUT PASTEUR

POSTDOCTORAL FELLOWSHIPS
Institut Pasteur, Paris, France

Founded in 1887 by Louis Pasteur and located in the heart of Paris, the Institut Pasteur is a world-renowned private research organization. The Pasteur Foundation of New York is seeking outstanding fellowship applicants. Candidates may apply to any laboratory within 10 Departments: Cell Biology and Infection, Developmental Biology, Genomes and Genetics, Immunology, Infection and Epidemiology, Microbiology, Neuroscience, Parasitology and Mycology, Structural Biology and Chemistry, and Virology. See website for details. Annual package is $70,000 for three years. This is a biannual call for applicants; see website for deadlines. *U.S. citizenship required.*

E-mail: pasteurus@aol.com. Website: http://www.pasteurfoundation.org.

POSTDOCTORAL RESEARCH FELLOWSHIP
in Blood Cell Biology
Joint Program in Transfusion Medicine
Harvard Medical School Affiliated Institutions
Boston, Massachusetts

Applications are now being accepted for Postdoctoral (M.D. or Ph.D.) Fellows who desire research careers in the expanding, multidisciplinary field of blood cell biology. The Program offers a stimulating environment with various basic science and applied research opportunities, and is supported by senior faculty in the Departments of Pediatrics, Pathology, Hematology/Oncology, and Genetics. Fellows will conduct independent research for two years under the direction of one mentor, with opportunities for collaborative and interlaboratory projects. The principal research fields include stem cell biology including immune modulation, gene therapy, cancer immunology, platelet biology, and hematopoiesis. Publication in an international peer-reviewed journal is a prerequisite for acceptance into the Program. As per NIH guidelines, *candidate must be a citizen of the U.S. or hold permanent residency status in the U.S. to apply.* Send curriculum vitae, statement of interest, and three reference letters to: **Leslie E. Silberstein, M.D., Director, Joint Program in Transfusion Medicine, c/o Blair Owren, Children's Hospital, Boston, Karp Family Research Building, 10th Floor, Room 10217, 1 Blackfan Circle, Boston, MA 02115** or via **e-mail: blair.owren@childrens.harvard.edu.**

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CHILDREN'S HOSPITAL BOSTON

The Division of Respiratory Diseases, Children's Hospital and the Department of Pediatrics, Harvard Medical School is seeking a scientist (M.D. and/or Ph.D.) who is interested in the study of inflammation. Salary and rank commensurate with experience. A strong publication record in the area of inflammation is required. Please send resume and names of references to: **Dr. Craig Gerard, Chair, Pediatric Respiratory Search Committee, 300 Longwood Avenue, Boston, MA 02115.** *Children's Hospital is an Equal Opportunity Employer. Women and minorities are encouraged to apply.*

POSTDOCTORAL POSITIONS available immediately at the newly established NanoMedicine Center at the University of Maryland **(website: http://www.pharmacy.umaryland.edu/nanomedicine/).** Candidates should have background in the following areas: (i.) nuclear magnetic resonance/MRI; (ii.) rheology; (iii.) small-angle scattering of x-rays and neutrons. Send curriculum vitae and a one-page research statement to **e-mail: byu@rx.umaryland.edu.**

POSITIONS OPEN

POSTDOCTORAL POSITIONS
Thermoregulation and Inflammation

We are looking for **POSTDOCTORAL FELLOWS/RESEARCH ASSOCIATES** to study lipid mediators of fever and hypothermia in systemic inflammation, physiological roles of transient receptor potential channels, and behavioral thermoregulation in rats and mice. Background in systems physiology, molecular biology, or immunohistochemistry/neuroanatomy is preferred, but the ability to think and work independently, dedication to work, and persistence in the face of failure are more important than the area of specialization. A specific direction of research will be determined by the Laboratory director to closely match the line of expertise and interests of each successful candidate. Mandatory requirements include an advanced degree, a track record of peer-reviewed publications, excellent computer skills, and good writing skills. Recent publications from the Laboratory include *Public Library of Science Biology* **4**:284, 2006, and *ONE* **1**:1, 2006, published online. Mail your curriculum vitae, reprints of full-length papers, a brief description of research interests and career goals, and names, e-mail addresses, and telephone numbers of at least two references to: **Andrej A. Romanovsky, M.D., Ph.D., Director, Systemic Inflammation Laboratory, St. Joseph's Hospital, 350 W. Thomas Road, Phoenix, AZ 85013 U.S.A.** *Affirmative Action/Equal Opportunity Employer.*

DIRECTOR for MUSCLE and AGING RESEARCH UNIT
Boston Medical Center

Ph.D. scientist with five years of postdoctoral experience in basic muscle biology/stem cells/satellite cells to head a translational muscle biology and anabolic drug discovery research group; record of publication required; attractive salary and support package; appointment at **ASSOCIATE PROFESSOR** level possible. Please e-mail curriculum vitae to **e-mail: ana.mercurio@bmc.org** or call **Ana Mercurio** at **telephone: 617-414-1833** if you would like more information.

POSTDOCTORAL POSITION available to study the genetics, molecular and cell biology of aging in yeast (e.g. **Seo et al.,** *Aging Cell* **6**:405, 2007). Applicants should have training/experience in one of the above areas and facility with recombinants DNA. Send curriculum vitae and names and e-mail addresses of three references to: **S. Michal Jazwinski (e-mail: sjazwi@lsuhsc.edu), Department of Biochemistry and Molecular Biology, Louisiana State University Health Sciences Center, 1901 Perdido Street, New Orleans, LA 70112 U.S.A.** *Affirmative Action/Equal Opportunity Employer.*

MOLECULAR BIOCHEMIST to study transcription-induced hypermutation in human cell lines. Expertise in genetic engineering and cell culture techniques required. Position available this August. Send curriculum vitae to **e-mail: barbara.wright@mso.umt.edu,** University of Montana. *Affirmative Action/Equal Opportunity Employer.*

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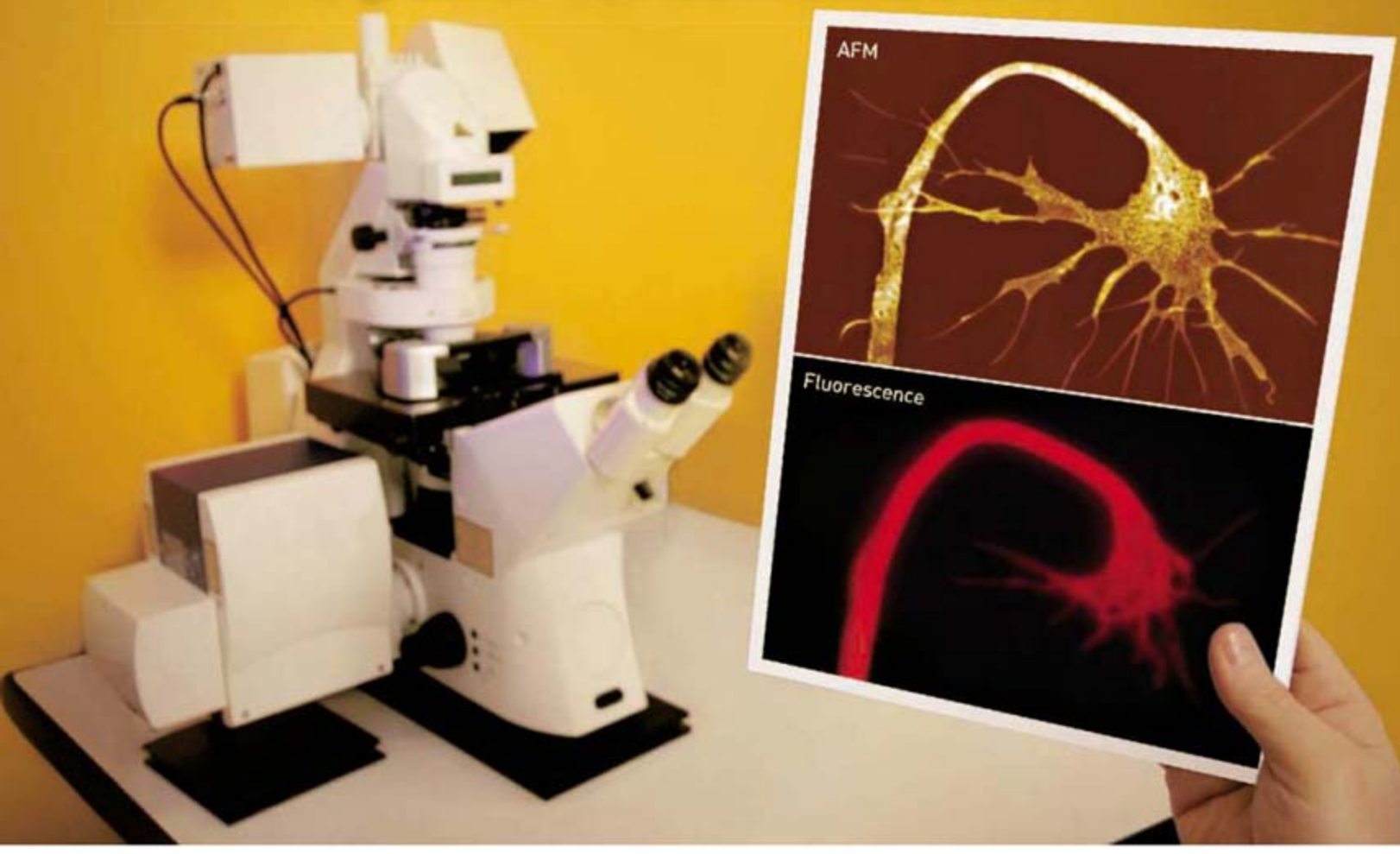

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